

FERTILITY
and
HATCHABILITY
of
Chicken and Turkey Eggs

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EDITED BY

Lewis W. Taylor

UNIVERSITY OF CALIFORNIA, BERKELEY

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FOREWORD

The International Baby Chick Association, the trade organization of the commercial and breeder hatchery industry, through its Board of Directors at their annual meeting in 1945 appointed a Research Committee to devise means of selecting, initiating, and carrying out research projects of value to the industry.

The Research Committee decided upon and set up a systematic research program to be implemented by funds allocated as fellowships and grants-in-aid to universities, colleges, and experiment stations where such research could be carried on.

The first subject to receive consideration by the committee was the rather general one of factors influencing hatchability and fertility. Studies on this subject would, of course, be of immediate and practical interest to an organization of hatchery-men.

Before initiating further research in this field the committee felt that a fairly comprehensive review of the literature on the subject should be compiled. Such a review would not only bring the industry up to date on the subject but also undoubtedly indicate specific areas where further research was needed. Poultry scientists who were consulted suggested also that such a review would fill a need as a reference and textbook in agricultural colleges.

This volume, which reviews all the significant literature on the factors influencing fertility and hatchability of chicken and turkey eggs, is, therefore, the result of co-operation between the International Baby Chick Association and the various poultry scientists who acted as collaborators. The Research Committee, on behalf of the hatchery industry, gratefully acknowledges the assistance and collaboration of the following scientists who have made this work possible: Dr. W. W. Cravens, Department of Poultry Husbandry, University of Wisconsin, Madison, Wisconsin; Dr. D. C. Warren, Department of Poultry Husbandry, Kansas State College, Manhattan, Kansas; Dr. J. E. Parker, Department of Poultry Husbandry, Oregon State College, Cor-

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Especial acknowledgment is made of the advice and assistance of Dr. Lewis W. Taylor, Division of Poultry Husbandry, University of California, Berkeley, California, who has edited these reviews and whose help in this and other ways has been immeasurable.

Further acknowledgment is made of the advice and co-operation of the reviewers whose names are listed in the preface. Their criticisms have greatly aided the editor and his collaborators in developing the contents of the various chapters.

It is hoped that this volume may be useful to the poultry industry, to students, and to others interested in poultry science.

E. A. NISSON

Chairman, Research Committee
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PREFACE

In accepting their assignments in the co-operative effort to prepare this review, the collaborating authors and the editor have recognized the difficulties involved in the translation of scientific findings into terms of practical application. They have recognized also that the readers of this book will vary widely in scientific training and in practical hatchery experience. Special effort has accordingly been made to explain in the text and to define in the glossary the various scientific and industrial terms used.

The authors have made an extensive survey of the experimental work reported in their respective scientific fields. The significant results and theories developed from such reports published and available to the collaborators by December, 1947, are included in the various chapters. No attempt has been made to cite all publications dealing with reproduction in chickens and turkeys. A list of pertinent references, with stress placed on papers and reviews giving comprehensive citations of literature, has been provided for students and investigators who may desire to make a detailed study of specific phases of fertility or hatchability. Particular consideration has been given to an evaluation of results obtained from past research and to the problems remaining to be solved.

It is the hope of all concerned in the preparation of this volume that their efforts will serve to promote education and research in the breeding and hatchery phases of the poultry industry.

Grateful acknowledgment is made to Dr. H. J. Almquist of the F. E. Booth Co., Emeryville, California, and to a group of colleagues of the editor from the University of California who have reviewed and criticized various chapters as follows: Chapter 1, Dr. Almquist and Dr. C. R. Grau; Chapters 2, 4, and 9, Dr. V. S. Asmundson; Chapter 3, Dr. F. W. Lorenz; Chapter 5, Dr. Grau; Chapter 7, Dr. I. M. Lerner; and Chapter 8, Dr. K. B. DeOme. Dr. R. M. Eakin also made valuable suggestions con-

Preface

cerning the program of graduate training discussed in Chapter 9.

The Research Committee and the Executive Directors of the International Baby Chick Association have generously met every request from the collaborating authors and the editor for aid in the preparation of this review. To them should be attributed an important share in whatever merit may be found in the contents of this volume.

L. W. T.

Berkeley, California

June, 1949

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The Nutrition of the Breeding Flock

by W. W. CRAVENS

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INTRODUCTION

Feeding the breeding flock is in essence feeding the embryo through the medium of the hen. It is thus essential to feed the hen so that her eggs will be richly endowed with all nutritive factors required for differentiation and growth of the embryo. A shortage in the egg of any one of the essential nutritive factors may result either in death of the embryo or in a chick or poult lacking vitality and therefore the ability to live and grow normally. Faulty nutrition of the breeding flock may, then, be a source of considerable loss to the poultry industry. In her excellent review Cruickshank (1941) has pointed out the direct relationship existing between hatchability and the quantity of certain nutrients in the egg.

Problems related to the feeding of the breeding flock have become more acute with the development of artificial incubation and the resulting "out-of-season" hatching. In addition, improvements in breeding, management, and feeding have resulted in the production of more eggs during the winter months. In sections of the country where green feeds are available most of the year, "out-of-season" hatching has been practiced for many years with fairly satisfactory results. But, for the country as a whole, most early attempts to hatch chicks during periods other than the normal hatching season met with failure, whereas satisfactory results were obtained when the birds were allowed access to pasture and sunlight. Considerable progress has been made in relatively recent years in determining both qualitatively and quantitatively the nutritive factors required by chickens for hatchability, but much remains to be done before our knowledge is complete.

For the purpose of this review it must be assumed that both the ration fed the breeding flock and the system of management practiced are satisfactory for egg production. Therefore little mention will be made of feeding for egg production alone. It is well recognized that satisfactory egg production may be obtained with rations that are inadequate for the production of hatchable eggs, although eggs so produced do not possess maximum nutritive value for the consumer.

In this chapter the basic problems of nutrition in relation to hatchability and, as far as possible, to fertility will be considered. The nutritive factors are considered largely in relation to stability in rations and to practical sources. Emphasis will be given to the relationship between a deficiency of a particular nutritive factor in the diet of the hen and the characteristic age at death or any symptoms exhibited by the embryo. Such information adequately determined for all nutritive factors may provide a method of diagnosing nutritive deficiencies in breeding flocks. No attempt will be made to suggest formulas for rations for the breeding flock since the feedstuffs available for compounding rations are so different in various sections of the country. The feedstuffs supplying unidentified factors will be stressed. The nutritive allowances suggested later are those given by the Committee on Animal Nutrition of the National Research Council. They represent a more accurate application to practical feeding conditions of the results of experimental studies on nutritive requirements of breeding hens than could be arrived at by any one individual:

CARBOHYDRATES

The effect of different carbohydrates on hatchability has not been studied to any extent. Couch, Cravens, Elvehjem, and Halpin (1947) found that the presence of dextrinized cornstarch in purified rations favored intestinal synthesis of biotin in contrast to little or no synthesis of the vitamin when sucrose served as the carbohydrate. The magnitude of synthesis was reflected in the biotin content of the egg and also in hatchability (fig. 1). Numerous studies dealing with the various grains in breeding rations have been reported, but obviously other factors than the carbohydrates are involved in such experiments. In general such studies have indicated that a mixture of cereal grains is superior to any single grain.

LIPIDS

The lipids include a wide variety of substances that are insoluble in water but soluble in ether, chloroform, and benzene. The fats make up the bulk of the lipids present both in the animal body and in the rations as well, but certain other lipids are important in nutrition. The lipids are particularly important

because of their intimate association with certain vitamins and with the antioxidants of feedstuffs. Very little study has been made of the lipids other than the fats in the nutrition of mature fowls.

Cruickshank (1934) demonstrated the relationship between dietary fat and the fat in egg yolk. It was found that the de-

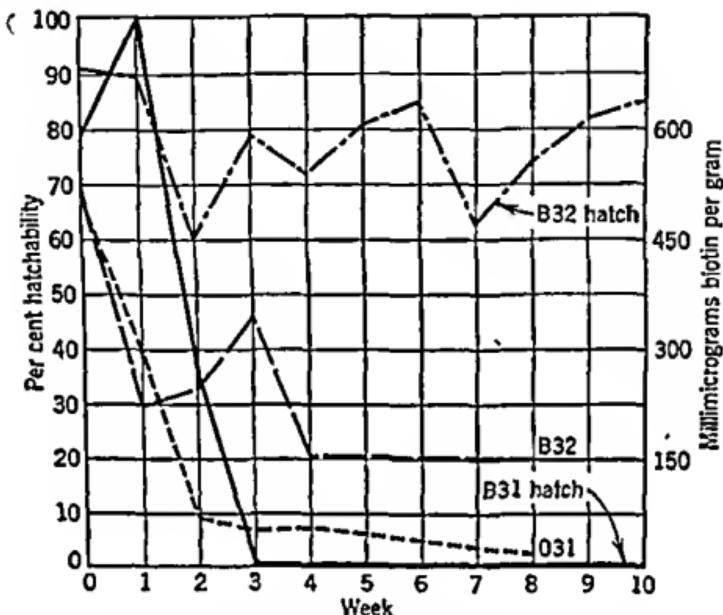


Fig. 1. Relation of dietary carbohydrate to biotin content of egg yolk and hatchability. B31 sucrose; B32 dextrin. — Hatchability; - - - millimicrograms biotin per gram of yolk. (From Couch, Cravens, Elvehjem, and Halpin, 1947.) Courtesy *Journal of Nutrition*.

gree of unsaturation, as measured by the iodine number, could be markedly increased by feeding a soft fat (rapeseed oil), whereas the feeding of a hard fat (mutton fat) failed to alter significantly the composition of egg yolk. McCollum, Halpin, and Drescher (1912) proved the synthesis of lecithin by the hen.

The effect of the level of dietary fat on egg production and hatchability has been studied by Heywang (1942). Varying the level of fat in the ration from less than 1 per cent to more than 8 per cent by adding corn oil did not significantly affect hatchability or the period of embryonic mortality. Similar results have been reported by Taylor, Jeffrey, and Russell (1944). On

the other hand, Ringrose, Morgan, and Lease (1941) report a marked reduction in hatchability by the addition of 3.6 per cent of crude cottonseed oil to the diet of breeding hens. Crude soybean oil and peanut oil were without effect. These workers report that the active factor was present in the saponifiable fraction of the cottonseed oil and that, although it was stable to heating and oxidation, it was destroyed by hydrogenation. These results indicate that the detrimental effects of cottonseed oil are due to a toxic substance characteristic of this fat.

The relation between the fat content of the diet and the absorption of vitamin A and carotene by the hen has been studied by Russell, Taylor, Walker, and Polskin (1942). Rations extremely low in fat were found to decrease the efficiency of carotene absorption but that of vitamin A was not affected.

From these studies it seems safe to conclude that the level of fat likely to be present in breeding rations composed of natural ingredients will not influence hatchability. The importance of fats in poultry-breeding rations should not be minimized, however, because of their relation to the fat-soluble vitamins and to the antioxidants present in feedstuffs. Many studies have demonstrated the destructive nature of rancid fats on certain vitamins, particularly on vitamins A (Lease, Lease, Weber, and Steenbock, 1938), E (Evans and Burr, 1927), and biotin (Paveck and Shull, 1942).

PROTEINS

The proteins characteristically yield alpha amino acids on hydrolysis; many proteins, however, are combined with non-protein substances in nature and are thus classified as conjugated proteins. In nutrition the value of a protein is dependent upon its amino acid make-up, but in actual feeding practice the value of the so-called protein feeds may be due in part to the presence of many substances other than protein. Since such feeds have been used in most experiments designed to determine the effect of proteins on hatchability, we have little experimental data on which to base any conclusions regarding the effect of proteins, or more specifically the amino acids, on hatchability.

The effect of the diet fed the breeding hen upon the amino

acid make-up of eggs has been the subject of several investigations. McFarlane, Fulmer, and Jukes (1930) found no relation between the proteins in the diet fed and the tyrosine, tryptophane, cystine, total amino nitrogen, or total nitrogen content of eggs. Titus, Byerly, and Ellis (1933) found a slight but significant increase in the per cent of protein in the dry matter of egg yolks that was attributable to the ration fed the hen. Calverry and Titus (1934), however, reported no appreciable difference in the composition of the proteins of eggs from hens fed proteins from wheat, corn, or soybean oil meal. Similar results were reported by Patton and Palmer (1936). These workers also presented evidence indicating that glycine is synthesized during embryonic development. It was found, however, that the glycine content of chondrodstrophic embryos was lower than that of normal embryos; hence certain factors may influence the deposition of protein in the embryo during development. Grau (1947) has shown that the lysine content of eggs was not modified by the feeding of diets of low lysine content to the laying hen.

The effect on egg production of a combination of casein and gelatin in purified rations was reported by Cravens and Halpin (1946). Although egg production dropped precipitously when hens were fed the basal ration, hatchability apparently was not affected. In a later study Cravens (1947) found that the addition of leucine, methionine, and tryptophane to the basal ration prevented the rapid decrease in egg production. This preliminary evidence indicates that the amino acids as such may not affect hatchability since egg production ceases when there is an amino acid deficiency.

Numerous studies have been conducted on the relative merits of various protein feedstuffs for breeding hens. Since such studies are complicated, as we have indicated, by the presence of nutritional factors other than the proteins in the feedstuffs employed, they will be discussed in this review under the heading of feeding practices. An excellent review of proteins in poultry nutrition has been published by Hill (1944).

It should be pointed out, however, that there is a fertile field for future research in studies on the amino acid requirements of hens for egg production and hatchability.

VITAMINS

Since 1920 the role of the vitamins in hatchability has received considerable study. Certain vitamins are relatively unstable, and thus special consideration must be given to them in poultry feeding. The vitamins are also of practical importance because the breeding hen has rather high requirements for certain ones, and thus rations compounded of practical feedstuffs may not supply these in adequate quantities for hatchability.

The vitamins, because of their chemical properties, are generally divided into the fat-soluble and water-soluble groups. The fat-soluble group includes vitamins A, D, E, and K, whereas ascorbic acid and the vitamins of the B-complex belong to the water-soluble group. In general, if the diet contains more than the bird's needs, the fat-soluble vitamins are stored in the body in quantities sufficient to protect the animal against deficiency symptoms for a considerable period, whereas depletion of the water-soluble vitamin reserves of the body may occur quite rapidly.

Vitamin A

The needs of mature fowl for vitamin A were established by Beach (1924). Since that time numerous reports have indicated the essential nature of vitamin A for egg production and hatchability.

Vitamin A does not occur as such in plant materials but appears in the form of certain carotenoid pigments, its precursors, which are converted into the vitamin in the animal body. The most abundant of such pigments are carotene from green feeds and cryptoxanthin from yellow corn. These compounds are often referred to as provitamins A. The fish oils are the chief sources of true vitamin A in breeding rations.

Since certain carotenoid pigments are converted to vitamin A in the body of the hen and transferred to the egg yolk, their metabolism is of considerable importance. The chief coloring matter in egg yolk is xanthophyll which does not function as vitamin A. The laying hen selectively transfers xanthophyll to the egg yolk along with smaller quantities of carotene and crypto-

xanthin. An excellent study and review of the metabolism of carotenoid pigments by the hen was published by Peterson, Hughes, and Payne (1939).

The transfer of pigments from the hen's diet to the shanks of the chick is an important practical consideration because deeply pigmented shanks are regarded as desirable. Hammond, Miller, and Whitson (1942) have reported that 3 per cent of fortified cod-liver oil or small quantities of sulfur fed the breeding hen suppressed pigmentation in the shanks of chicks, and Rubin and Bird (1946) have indicated that high levels of vitamin A suppress pigmentation. It is well known that chicks practically devoid of carotenoid pigments may be produced by hens that have been fed a diet free of these pigments.

Since certain carotenoid pigments serve as vitamin A precursors, the efficiency of their conversion to vitamin A by the fowl is a problem of considerable importance. Studies by Record, Bethke, and Wilder (1937) and by others indicate that the chick utilizes carotene as efficiently as the rat. Almquist, Mackinney, and Mecchi (1943) have shown that feeding hens equivalent practical dietary levels of vitamin A and carotene leads to an equivalent deposition of vitamin A potency in the egg (0.6 microgram of β carotene is equivalent to 1 International Unit of vitamin A). Baumann, Semb, Holmes, and Halpin (1939) have suggested that the developing embryo utilizes considerable vitamin A during incubation since less vitamin A was found in the day-old chick than in the unincubated egg.

Vitamin A and its precursors are readily destroyed by oxidation. The rate of destruction in the feed depends on such factors as temperature, the presence of certain minerals, particularly iron and copper, and the amount of surface exposed to the air. As mentioned previously, rancid fats cause a rapid destruction of vitamin A and its precursors. Wilder and Bethke (1941) have reported that the loss of carotene in machine-dried alfalfa may be as high as 60 to 70 per cent in 6 months of storage, and Baird, Ringrose, and MacMillan (1939) also noted a progressive destruction with storage of vitamin A from fortified cod-liver oil in a mixed feed. Fraps, Meinke, Reiser, and Sherwood (1943) found that certain animal-protein feedstuffs had the power of destroying carotene whereas feedstuffs of vegetable origin seldom pos-

sessed this property. These and numerous other studies serve to emphasize the importance of supplying the breeding flock with freshly mixed feeds.

The effect of varying quantities of vitamin A or provitamin A on egg production, hatchability, and chick livability has been studied by a number of workers. These studies have been reviewed by Sherwood (1939). Later studies on this subject have been considered by the Subcommittee on Poultry Nutrition of the National Research Council (1944) in arriving at the recommended allowances given in table 2 (page 38). The pronounced effect of the vitamin A content of the hen's diet on the vitamin A reserves of day-old chicks is emphasized by the experiments of Bearse and Miller (1937), Almquist and Mecchi (1939), and Baumann *et al.* (1939). Adequate reserves of this vitamin are extremely important in determining the chick's initial start in life.

Studies dealing with the importance of the tocopherols in the utilization of vitamin A will be considered in the section dealing with vitamin E.

Vitamin D

The discovery of the importance of vitamin D supplements and the relation of sunlight to vitamin D in the avian species has had a profound influence on the methods of poultry keeping. Hughes, Payne, and Latshaw (1924) and Hart, Steenbock, Lepkovsky, Kletzien, Halpin, and Johnson (1925) first reported the essential nature of vitamin D for the production of hatchable eggs.

Vitamin D is unique in that it may be either supplied as such in the ration or made in the body of the bird by the action of sunlight or ultraviolet light on the vitamin D precursors present there.

Vitamin D is known to exist in several forms. A great deal of research on its chemistry has been stimulated by the differences in efficacy of the various types of vitamin D for the chick as compared with the rat. Massengalo and Nussmeier (1930) reported that it required many more rat units of vitamin D from irradiated ergosterol than from cod-liver oil to prevent rickets in the chick. Steenbock, Kletzien, and Halpin (1932) confirmed these findings and reported that 40 to 120 times as much vitamin D was required to prevent rickets in chicks when it was supplied

in the form of irradiated ergosterol as when supplied by cod-liver oil. Branian and Smith (1932) and Bethke, Record, Kick, and Kennard (1936) have demonstrated that vitamin D from irradiated ergosterol is not as effective as that from fish oils in promoting egg production and hatchability. These workers also demonstrated that extremely high levels of vitamin D were toxic to breeding hens and resulted in decreased egg production and hatchability. The margin of safety is rather wide, however, and the toxic properties of high levels of vitamin D should not be considered of practical importance.

Work by Bird (1944) and Boucher (1944) indicates that turkey poult do not utilize vitamin D from fish oils as efficiently as that from irradiated animal sterols. Studies by Singsen, Matterson, and Scott (1947) indicate that, as the quantity of inorganic or noncereal phosphorus in the diet is increased, the difference in efficacy of vitamin D from the two sources is reduced. No studies on this subject in relation to breeding turkeys have been reported.

That vitamin D is not stable under all conditions has been demonstrated by the work of Fritz, Archer, and Barker (1942) and Fritz, Halpin, Hooper, and Kramke (1942), who reported the loss of the vitamin to be rather rapid when it was mixed with minerals, dried whey, or certain sugars. Cereals and other absorbent carriers tended to protect the vitamin although a slow but definite loss was observed when vitamin D was added to mixed feeds. The effect of manganese sulfate on the vitamins A and D in a " premix" consisting of fish oil and bran was studied by Miller, Joukovsky, and Hokenstad (1942), and a rapid loss of vitamin D was reported. However, no measurable loss was observed when the manganese sulfate was added to the feed in a premix of minerals as it would be in actual practice. Motzok and Slinger (1947) have suggested that the rate of destruction depends on the type of vitamin D. They reported that there may be two types of vitamin D in cod-liver oil, one stable and the other unstable. Vitamin D₃, presumably from irradiated animal sterols, was found to be quite unstable. Numerous other studies on the stability of vitamin D under different conditions have been summarized by Ewing (1947). The best evidence indicates that the loss of vitamin D is an oxidative process and thus is speeded up by conditions which promote oxidation. It would

appear, therefore, that the addition of large quantities of pulverized minerals to the breeding ration should be avoided and the storage period held to the minimum.

Since the original reports on vitamin D as an essential factor for egg production and hatchability, numerous publications have appeared on problems that relate to this vitamin in the nutrition of mature fowl. The reader is referred to Heuser (1946) for a complete review of the literature on the subject.

Vitamin D is closely related to the assimilation and metabolism of calcium and phosphorus, and consequently a shortage of this vitamin results in thin-shelled eggs and a withdrawal of minerals from the skeleton of the hen. Hart *et al.* (1925) demonstrated that there is a reduction in the mineral content of the egg shell in the event of a vitamin D deficiency. They also found that the embryo contained nearly twice as much calcium when the hens received adequate vitamin D as when they were deficient. This led them to suggest that vitamin D is related to the ability of the embryo to transfer calcium from the shell. In a very thorough study, Insko and Lyons (1936) likewise found that embryos from hens deficient in vitamin D contained less calcium and phosphorus than embryos from hens receiving adequate amounts of the vitamin. The greatest differences noted were on the eighteenth and the nineteenth days of development. It was also found that the peak of mortality among embryos deficient in vitamin D occurred on the nineteenth day of incubation. The distribution of embryonic mortality observed by Insko and Lyons is shown in figure 2.

It is of interest to note that, when the breeding hen is fed cod-

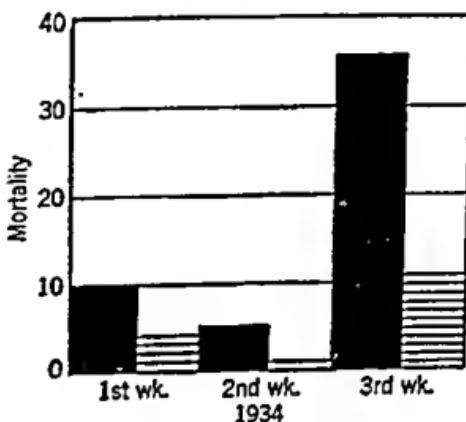


FIG. 2. The distribution by week of incubation of embryo mortality in two groups of White Leghorns which were confined to the pen. Shaded, 2 per cent cod-liver oil; black, no cod-liver oil. (From Insko and Lyons, 1936.)

Courtesy Kentucky Agr. Expt. Sta.

liver oil or is exposed to sunlight, an increase in iron and copper in her eggs results (Erikson, Boyden, Martin, and Insko, 1933). Chondrodystrophy has been reported to occur among embryos deficient in vitamin D, but it is probable that a type of nutritional micromelia was involved. The relation between vitamin D or sunlight and manganese in the nutrition of mature fowl reported by Couch, James, and Sherwood (1947) and Christiansen, Halpin, and Hart (1939) is of interest in this connection. Couch *et al.* found that less vitamin D was required in the presence of excess manganese, whereas Christiansen and his coworkers reported that sunlight or manganese prevented the low winter hatchability encountered with certain rations. Several other reports have indicated that sunshine supplies some factor other than vitamin D (Smith, 1933; Byerly, Titus, Ellis, and Nestler, 1937). Sunlight and, possibly, vitamin D may increase the efficiency of utilization of certain mineral elements other than calcium and phosphorus and some of the beneficial effects of sunlight may be indirect.

Somewhat conflicting results have been obtained in studies on the relation between the vitamin D content of the hen's diet and the growth rate and bone ash of the chicks. Bethke, Record, Wilder, and Kick (1936) reviewed the literature on this subject and presented data indicating that the vitamin D intake of the hen influences the growth and bone ash of chicks fed a ration deficient in this vitamin. In this study the most noticeable differences in storage of the vitamin in the chicks were observed at rather high levels of vitamin D intake by the hen. A similar carry-over has been reported in turkeys. Although it is doubtful that the growth rate of the chick or poult would be materially affected by the carry-over if their diet contained adequate amounts of vitamin D, a plentiful supply of the vitamin is essential for the breeder.

The best sources of vitamin D for breeding hens are sunlight, the fish oils, and activated animal sterols. The potency of these supplements for poultry is measured by a chick assay and is expressed in terms of A.O.A.C. (Association of Official Agricultural Chemists) units. One A.O.A.C. unit is equivalent in vitamin D activity to one I.U. (International Unit) contained in standard U.S.P. reference cod-liver oil.

Vitamin E (Alpha Tocopherol)

The necessity of vitamin E for embryonic development was demonstrated by the work of Card, Mitchell, and Hamilton (1930). In this experiment pullets were raised from 8 weeks to maturity on a ration treated with ferric chloride in ether solution to destroy vitamin E. The first setting of eggs was made after the birds had been fed the ration for approximately 8 months. Of 317 fertile eggs, only 41 developed beyond the ninth day of incubation and not a single chick hatched. When each bird was fed 0.5 cubic centimeter of wheat-germ oil daily an immediate improvement in hatchability resulted and after 2 weeks apparently normal hatchability was obtained. When such feeding was discontinued hatchability declined in 2 weeks practically to zero.

In a study of embryos from hens receiving the rations treated with ferric chloride, Adamstone (1931) observed marked retardation both in growth rate and in differentiation of tissues after 24 hours of incubation. The blood vascular system failed to form completely or degenerated shortly after being laid down. A lethal ring was found to develop in the vascular area outside the embryo. This ring often completely surrounded the embryo at 4 to 5 days of incubation and involved intensive cell proliferation in the mesoderm of the yolk sac. The entoderm of the sac was often completely disintegrated in regions where the lethal ring developed. The extra-embryonic circulatory system was choked off by the lethal ring. Hemorrhages and other disturbances in the circulatory system seemed to be the principal direct causes of death. The peak in embryonic mortality was found to occur after 84 to 96 hours of incubation. Barnum (1935) also noted a high embryonic mortality in the first week of incubation with vitamin-E-deficient hens. In addition the vitamin E content of the egg was found to be dependent upon the diet fed the hen.

Card *et al.* (1930) stated in their original report, "Although these results are of considerable scientific interest it should be pointed out that from a practical feeding standpoint there is little cause for concern with regard to possible vitamin E deficient rations because all whole grains and many green feeds are good

sources of this vitamin." The truth of this statement is supported by reports showing that the addition of wheat-germ oil to practical breeding rations is without benefit (Card, 1929; Holmes and Cravens, 1940). On the other hand, Ender (1935) reports an improvement in fertility and hatchability from the feeding of wheat-germ oil.

Nevertheless, the importance of alpha tocopherol and related compounds in practical nutrition should not be minimized. Moore (1940) demonstrated the antioxidant action of tocopherols in the animal. Considerable work along this line has shown that the biological response of vitamin A and carotene is greatly influenced by the presence of tocopherols and to a certain extent of other antioxidants in the ration. An excellent review of the function of vitamin E as a true vitamin as well as of the general function of the tocopherols in nutrition has been published by Hickman and Harris (1946).

Vitamin K

Dam in 1929 observed that chicks raised on certain diets became anemic, had large subcutaneous and intramuscular hemorrhages, and in one chick the clotting time of the blood was markedly prolonged. Later Dam (1935) reported that the hemorrhagic condition was not relieved by the addition to the diet of any known dietary factors, and the name vitamin K was suggested for the unidentified factor. Since these reports were made a vast amount of literature has accumulated concerning vitamin K. Almquist and Klose in 1939 reported that plthiocol possessed vitamin K activity. Shortly thereafter the synthesis of vitamin K₁ was accomplished, and numerous compounds have since been shown to possess vitamin K activity. Almquist (1941) has published an excellent review on the subject.

Vitamin K is present abundantly in green plants and is synthesized by certain microorganisms—a fact which resulted in some confusion in early attempts to isolate the vitamin. The vitamin is relatively stable though it is inactivated by light.

The relation of vitamin K to hatchability has not been studied thoroughly. Almquist and Stokstnd (1936) studied various factors influencing the incidence of vitamin K deficiency in chicks. They found that the quantity supplied to the hen markedly af-

fектs the reserves of the chicks at hatching time, as determined by survival time of the chicks. They also showed that vitamin K is synthesized in the intestinal tract of the chick and that further synthesis of the vitamin occurs in the droppings after voiding. Cravens, Randle, Elvchjem, and Halpin (1941), after observing fatal hemorrhages in chicks as a result of wing banding, studied the relation between the quantity of green feeds in the hen's diet and the clotting time and prothrombin time of the day-old chicks. That a direct relationship exists is indicated from the data in table 1.

TABLE 1

EFFECT OF VITAMIN K, SUPPLIED BY GREEN FEEDS, IN THE HEN'S DIET ON BLOOD CLOTTING AND PROTHROMBIN TIME OF DAY-OLD CHICKS

Hen group	Source of vitamin K in hen's diet		Measure of carry-over from dam to chick	
	Alfalfa leaf (percentage)	Dried cereal grass (percentage)	Blood-clotting time (minutes)	Pro-thrombin time (seconds)
1	0	0	11.6	40.0
2	0.8	0	6.7	31.8
3	2.0	0	4.6	24.9
4	4.0	0	5.1	21.3
5	0	0.4	7.2	28.5
6	0	0.8	6.3	26.1
7	0	2.0	5.5	22.8

Although no systematic study was made, Thayer, McKee, Binkley, MacCorquodale, and Doisy (1939) reported: "Our experience indicates that the degree of deficiency in different lots of chicks varies considerably, but that the main variation seems to be seasonal. During the late winter and early spring the deficiency is greatest."

From these studies it would appear that, even though vitamin K is synthesized in the intestinal tract of mature fowl, this synthesis and subsequent absorption does not proceed at a rate commensurate with the needs of the breeding hen. The importance of including a good source of vitamin K in the hen's diet is thus indicated, but the available data do not establish whether or not this vitamin is essential for embryonic development.

Riboflavin

Shortly after the role of vitamins A and D in hatchability had been established, it became apparent to many investigators that there was also present in such materials as green feeds, liver, milk, and yeast a factor (or factors) necessary for embryonic development (Bethke and Kennard, 1928; McFarlane *et al.*, 1930; Titus *et al.*, 1933). The requirement of the growing chick for both a

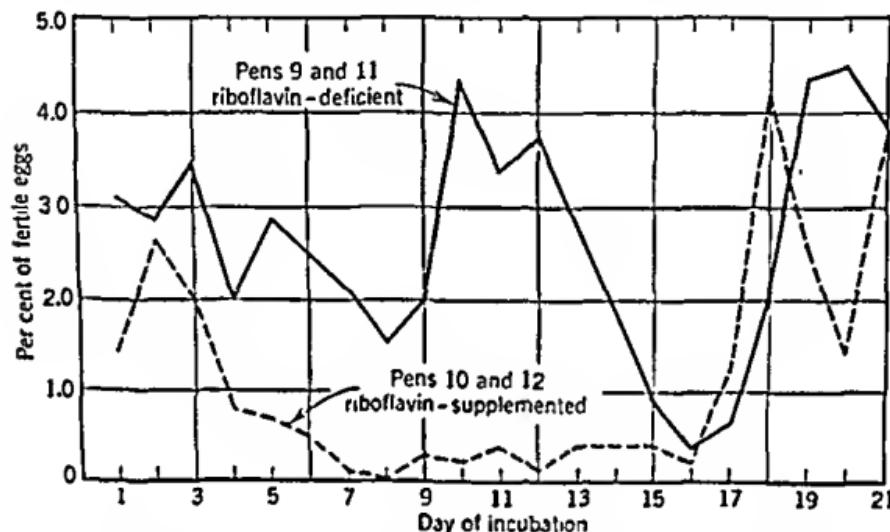


Fig. 3. Distribution of embryonic mortality on rations containing adequate and inadequate riboflavin. (From Lepkovsky, Taylor, Jukes, and Almquist, 1938.) Courtesy California Agr. Expt. Sta.

fairly low in this vitamin. Good-quality green feeds, milk products, certain fermentation products and residues, yeast, and liver meal are good sources of riboflavin. It should be emphasized, however, that even these feeds vary widely in riboflavin content, and thus no generalizations can be made regarding the quantity

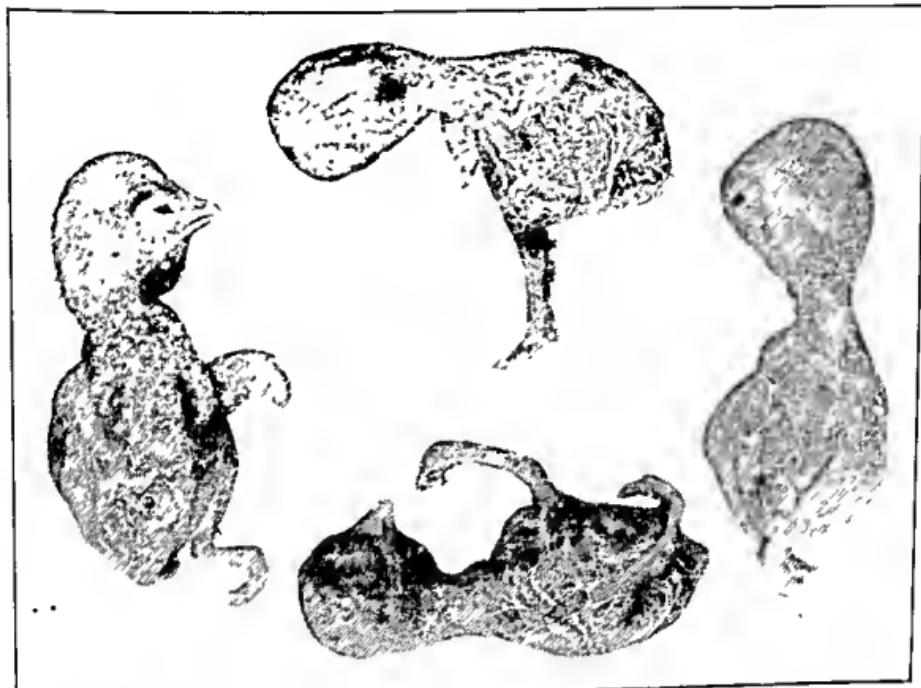


FIG. 4. Gross symptoms of embryos from riboflavin-deficient hens. Note clubbed down and generalized edema. (From Lepkovsky, Taylor, Jukes, and Almquist, 1938.) Courtesy California Agr. Expt. Sta.

that should be used to meet the needs of the breeding hen. Only by knowing the amount of the vitamin present in the feed and the requirements of the bird can one determine how much of the feed is required to supplement the riboflavin in the basal mixture.

Riboflavin is stable except in the presence of light. Even in mixed feeds the losses encountered should not be a major practical problem since Stemberg and Peterson (1946) have shown that when a mixed feed was exposed to direct sunlight, and stirred several times each day, only about 10 to 14 per cent of the riboflavin present was destroyed in 9 days. Such conditions would seldom be encountered in actual feeding practice.

The influence of the riboflavin content of the hen's diet on hatchability, age at death of the embryo, abnormalities of the embryo, and carry-over to the chick has received considerable study. Lepkovsky, Taylor, Jukes, and Almquist (1938) observed a marked increase in embryonic death during the mid-period of

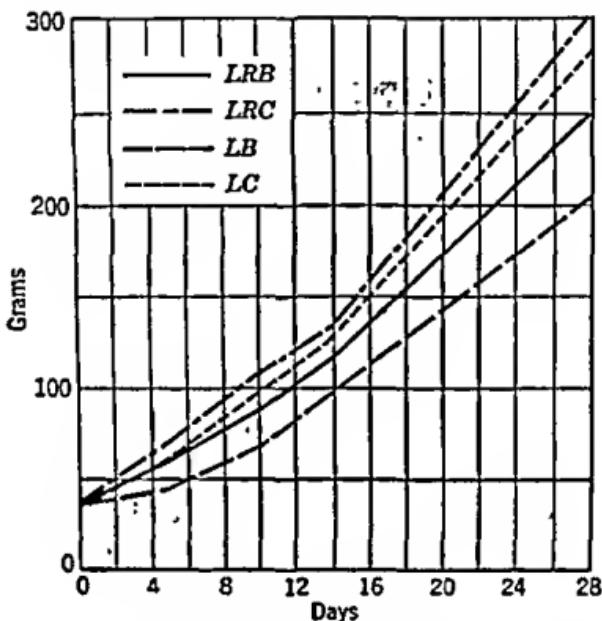


FIG. 5. Effect of varying amounts of riboflavin in the hen's diet on growth of chicks receiving rations of different riboflavin content. LB: low breeder ration; low chick mash. LC: low breeder; inadequate chick. LRB: inadequate breeder; deficient chick. LRC: adequate breeder; adequate chick.

(From Clandinin, 1946.) Courtesy *Poultry Science*.

development to be associated with riboflavin deficiency (fig. 3). These workers also noted that riboflavin eliminated or reduced the incidence of anaemia, edema, defective or clubbed down, degeneration of Wolffian bodies, and dwarf-type embryos. The clubbed down and edematous conditions are illustrated in figure 4. Romanoff and Bauernfeind (1942) essentially confirmed these results, although they reported a higher mortality during the first critical period, as had also Davis, Norris, and Heuser (1938). A general micromelia, or shortening of the extremities, was also observed. Considerable difference was noted in the rate at which different hens were depleted of riboflavin. Although the tech-

nique used in grouping the birds may be questioned, Romanoff and Baucrnfieind suggested that the peak of embryonic mortality shifts from toward the end of the incubation period to nearer the beginning in accordance with the degree of depletion of the mother hen. Engel, Phillips, and Halpin (1940) found that degeneration of the myelin sheath occurred in the sciatic nerve of about 60 per cent of the embryos from a group of hens fed a riboflavin-deficient diet. Curled toes, a characteristic symptom of riboflavin deficiency in the growing chick, has been reported to occur in day-old chicks hatched from riboflavin-deficient hens.

Numerous studies have indicated that the quantity of riboflavin fed the breeder hen influences the rate of growth of the progeny (Norris, Wilgus, Ringrose, Heiman, and Heuser, 1936; Lepkovsky *et al.*, 1938). In a more recent study of this relationship, Clandanin (1946) reported that the riboflavin reserves of the chick at hatching are dependent on the quantity of this vitamin fed the breeding hen. The riboflavin reserves at hatching time were also found to be closely related to growth and to the incidence of curled-toe paralysis during the chick's first 4 weeks of life. The important relationship existing between the riboflavin fed the breeder hen and the growth of the progeny when fed rations of different riboflavin content is illustrated in figure 5.

Pantothenic Acid

The necessity of an unidentified factor for the prevention of dermatitis in chicks was suggested by Ringrose, Norris, and Heuser (1931). Kline, Keenan, Elvehjem, and Hart (1932) found that extended dry heating of a ration composed of crude ingredients destroyed a factor or factors essential for the prevention of a dermatitis in chicks. This factor was termed the chick antidermatitis or antidermatosis factor by certain investigators, and because of its chemical properties it was termed the filtrate factor by others. A factor called pantothenic acid was at the same time being extensively studied as a growth factor for certain microorganisms (Williams, 1941). The identity of the chick antidermatitis factor with pantothenic acid was established almost simultaneously by Woolley, Waisman, and Elvehjem (1939) and Jukes (1939).

The influence of the riboflavin content of the hen's diet on hatchability, age at death of the embryo, abnormalities of the embryo, and carry-over to the chick has received considerable study. Lepkovsky, Taylor, Jukes, and Almquist (1938) observed a marked increase in embryonic death during the mid-period of

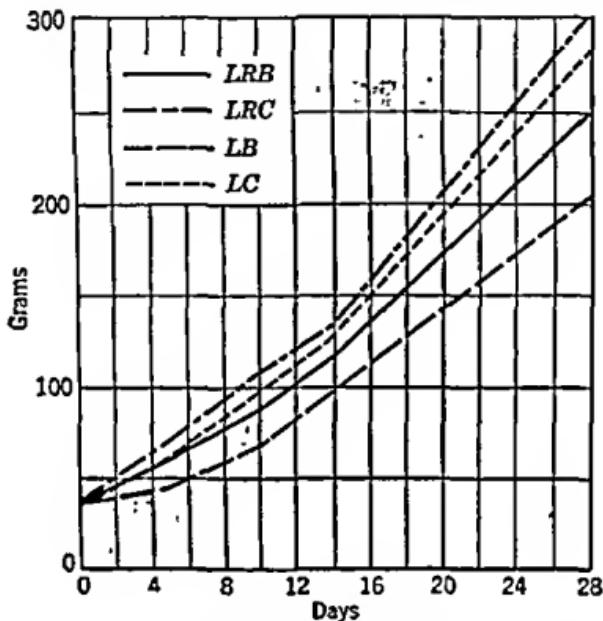


FIG. 5. Effect of varying amounts of riboflavin in the hen's diet on growth of chicks receiving rations of different riboflavin content. LB: low breeder ration; low chick mash. LC: low breeder; adequate chick. LRB: adequate breeder; deficient chick. LRC: adequate breeder; adequate chick.

(From Clandinin, 1946.) Courtesy *Poultry Science*.

development to be associated with riboflavin deficiency (fig. 3). These workers also noted that riboflavin eliminated or reduced the incidence of anemia, edema, defective or clubbed down, degeneration of Wolffian bodies, and dwarf-type embryos. The clubbed down and edematous conditions are illustrated in figure 4. Romanoff and Bauernfeind (1942) essentially confirmed these results, although they reported a higher mortality during the first critical period, as had also Davis, Norris, and Heuser (1938). A general micromelia, or shortening of the extremities, was also observed. Considerable difference was noted in the rate at which different hens were depleted of riboflavin. Although the tech-

Biotin

The reader is referred to Hertz (1946) for a complete review of the literature dealing with studies that led to the isolation, synthesis, and establishment of biotin as a nutritive essential for

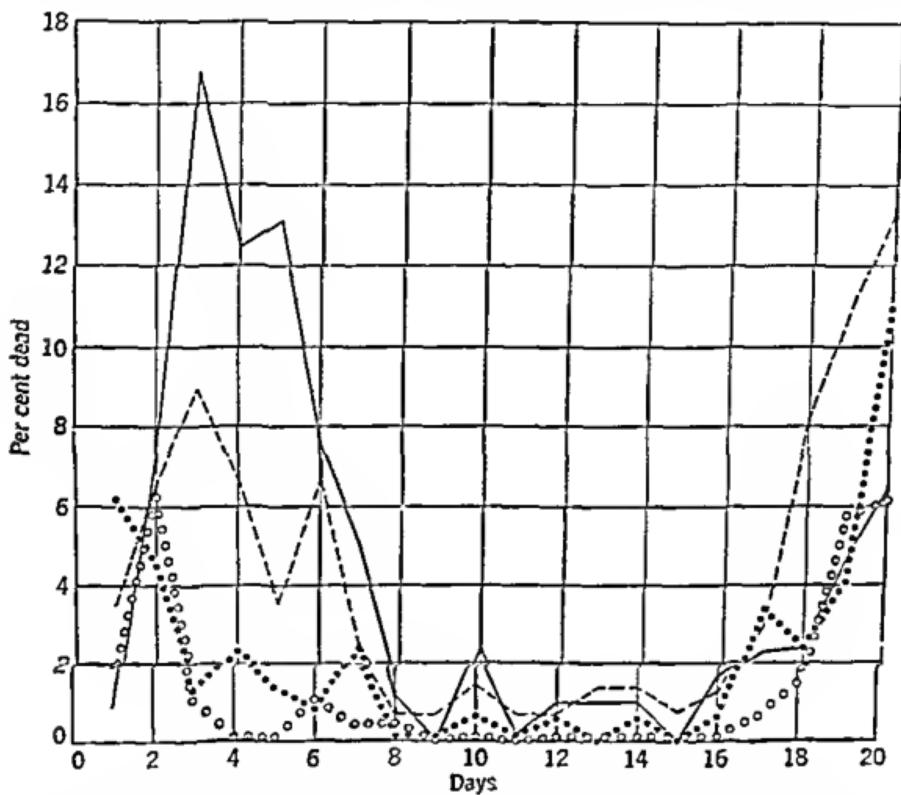


FIG. 6. Distribution of embryo mortality on rations containing varying amounts of biotin. Solid line indicates basal diet only; broken line, basal plus 30 micrograms of biotin per kilogram of ration; dotted line, basal plus 60 micrograms; circles, basal plus 150 micrograms. (From Cravens, McGibbon, and Sebesta, 1941.) Courtesy Anatomical Record.

microorganisms and animals. The essential nature of biotin for hatchability was demonstrated by Cravens, Sebesta, Halpin, and Hart (1942a).

The first study of the role of pantothenic acid in the nutrition of breeding birds was conducted by Lepkovsky *et al.* (1938). These workers were unable to demonstrate the necessity of the vitamin for hatchability although a definite relationship between the level of pantothenic acid in the diet of the hen and that in the eggs was established. Bauernfeind and Norris (1939) using a heated diet demonstrated that pantothenic acid is essential for hatchability. The heated diet has since been extensively employed by Norris and coworkers to study the role of pantothenic acid in egg production and hatchability, but considerable difficulty has been encountered in these studies because of the destructive effect of prolonged dry heating on other nutritive factors required by the breeding hen. The report, by Gillis, Heuser, and Norris (1947), on the quantitative needs of the breeding hen for pantothenic acid indicate that approximately 5.0 milligrams per pound of feed is adequate. Therefore, there is only a remote possibility of a deficiency of this vitamin in practical breeding rations since pantothenic acid is quite widely distributed in common feeds and is stable under ordinary conditions. The green feeds and the outer covering of the cereal grains are excellent sources of this vitamin.

Pearson, Melass, and Sherwood (1945) have shown that pantothenic acid is not destroyed during embryonic development and that the quantity present in the newly hatched chick is dependent upon the amount in the egg. This same relationship was demonstrated earlier by Gillis, Heuser, and Norris (1943). These authors found that the chicks hatched from hens deficient in pantothenic acid were decidedly inferior in quality. The chicks usually exhibited general debility, muscular in-co-ordination, swollen hocks, and poor down quality. The peak of embryonic mortality was found to occur near the end of the incubation period; in fact 79 per cent of all embryonic deaths occurred after the fourteenth day, and 52 per cent after the eighteenth day of incubation. Taylor, Thacker, and Pennington (1941) observed a relationship between the pantothenic acid content of the eggs and the growth of certain organs of the embryo, as well as the concentration of other vitamins in certain tissues of these embryos. The significance of these latter findings is not apparent.

hatched from hens deficient in biotin. Perosis in the embryo and in the day-old chick has also appeared when the hen is deficient in biotin. The ataxia was characterized in some chicks by head retractions and in others by the head being drawn ventrally in a posterior direction. Continuous involuntary rotational movements of the head persisted until death. The legs were extended to the rear, the hocks were stiff, and the chick was unable

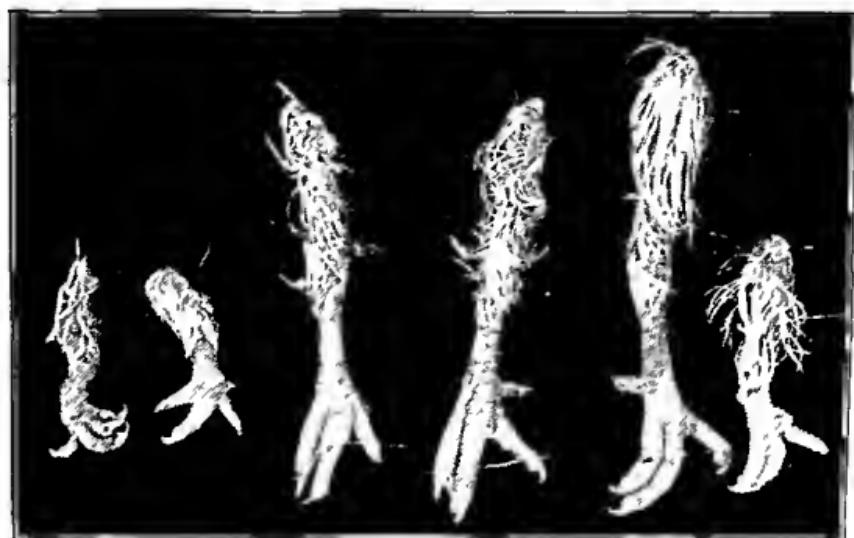


FIG. 8. Syndactyly or webbing of the toes resulting from a biotin deficiency. (From Cravens, McGibbon, and Sebesta, 1944.) *Courtesy Anatomical Record.*

to draw the legs up under the body so as to assume a standing position. In some chicks there was a marked spasticity and extension of the toes, and in others the toes were drawn to the rear (fig. 7). These symptoms in the day-old chick were not relieved by the oral administration or injection of biotin.

Studies to date do not allow definite conclusions regarding the possibility of practical breeding rations being inadequate in this vitamin. It is of interest that symptoms similar to those encountered in a biotin deficiency have appeared among embryos from hens fed rations containing soybean oil meal as the sole protein supplement (Cravens, 1947). A deficiency of biotin has also been encountered when similar rations were fed to rats (Spitzer and Phillips, 1946). Whether such results indicate a true defi-

of development. A moderate biotin deficiency resulted in a lower peak at the first critical period, that is, from 3 to 4 days, but in a higher peak at the third period or from about the eighteenth to twenty-first day of incubation. Certain skeletal abnormalities also were observed. Numerous embryos exhibited a parrot-beak condition which was often associated with severely crooked tibiae



FIG. 7. Gross symptoms of embryos from biotin-deficient hens. Note crooked tibia, short, twisted tarsometatarsus, and parrot beak. (From Cravens, McGibbon, and Sebesta, 1944.) Courtesy Anatomical Record and the Wisconsin Agr. Expt. Sta.

and/or much shortened and twisted tarsometatarsi. Such embryos were termed chondrodystrophic but, since Landauer (1936) has differentiated chondrodystrophy from a nutritional micromelia by means of histological techniques, it seems advisable to use the more general term of nutritional micromelia for such embryos until histological studies may be made. Syndactyly, or extensive webbing between the third and fourth toes, also was observed. This condition has been reported in certain genetic studies but with conflicting evidence concerning its mode of inheritance (see chap. 7). Embryos exhibiting biotin-deficiency symptoms are shown in figure 7, and syndactylous toes in figure 8. In addition to the symptoms described above, Couch and Cravens (1947) have observed an ataxia in day-old chicks

by Lucas, Norris, and Heuser (1946) and Ringrose and Davis (1946). In neither of these studies were the investigators able to demonstrate the needs of mature fowl for choline. The apparent synthesis of choline by the hen was indicated by the latter investigators from the choline analyses they made of the eggs. Certain methylatable choline precursors may be utilized by the hen for the formation of choline. That such precursors are effective in assuming certain of the functions of choline for the chick has been suggested by a number of workers (see Almquist, 1946). Bethke, Kennard, and Chamberlin (1946) have presented data indicating that the addition of choline to diets composed of natural feedstuffs resulted in a decrease in hatchability. Similar results have also been obtained by other workers. Further work on the role of choline and other methylating agents in the nutrition of the adult fowl seems desirable in view of the conflicting results reported. It is obvious, however, that the addition of choline to practical breeding rations is unwarranted and may actually be detrimental.

Nicotinic Acid (Niacin)

The role of nicotinic acid in the nutrition of mature fowls has received little study. Dann and Handler (1941) and Snell and Quarles (1941) have demonstrated that nicotinic acid is synthesized by the developing chick embryo. Similar results have been obtained with turkey embryos (Furman, Snell, and Cravens, 1947). On the other hand, Briggs, Groeschke, and Lillie (1946) have reported that nicotinic acid is essential for egg production and hatchability. In this experiment high levels of bone ossein were fed since several workers had demonstrated that the feeding of high levels of proteins low in tryptophane alters the requirement of growing animals for nicotinic acid. The initial observations on this interesting relationship were made with rations low in protein and high in corn (Krehl, Tepley, Sarma, and Elvehjem, 1945). Although corn is a poor source of niacin, this vitamin is present in relatively large amounts in the bran layers of other cereal grains. Further work will be required to determine the needs for nicotinic acid of breeding hens under various practical dietary regimens.

ciency or are due to some other mechanism is not known at the present time, and further work on the role of this vitamin in breeding rations is thus indicated. The effect of different sources of carbohydrates on intestinal synthesis of biotin has been discussed previously (see carbohydrates).

Pyridoxin

Pyridoxin was shown to be essential for growing chicks by Carter and O'Brien (1939). Cravens, Sebesta, Halpin, and Hart (1943) showed that pyridoxin was essential for egg production and suggested that it was also a factor required for embryonic development. In a later paper the same authors, Cravens *et al.* (1946), established the approximate requirements of the breeding hen for pyridoxin. In these experiments so few eggs were obtained for incubation that the definite effect of a pyridoxin deficiency on hatchability was not obtained. In fact, the authors suggested that the effect observed may have been indirect because of the rapid decline in the general condition of the birds. No specific embryonic symptoms were observed. Further experiments will be required to determine more precisely the effects of pyridoxin on embryonic development. Rations composed of natural feedstuffs should supply adequate quantities of this vitamin.

Choline

McCollum, Halpin, and Drescher (1912), feeding a diet of skim-milk powder and polished rice, reported the synthesis by the laying hen of lecithin, a lipid containing choline. With essentially the same diet, although it was more adequately supplemented with vitamins that were unknown at the time of the work of McCollum *et al.*, Abbott and DeMasters (1940) reported that a dietary source of choline is essential for mature fowl. These investigators found that the rice-skim-milk diet would not support egg production or maintain body weight and that in addition there occurred large numbers of aborted yolks and fatty livers, the latter being also a symptom of choline deficiency in growing animals. The addition of choline prevented these symptoms.

Since the observations of Abbott and DeMasters, data on the effect on laying birds of diets low in choline have been presented

as by inferior quality of the chicks hatched. Almquist (1943) had previously reported that a concentrate of the *L. casei* ϵ factor in the diet of the hen increased growth and improved survival of the chicks. Insufficient data are available to evaluate practical breeding rations for this vitamin, though many of the common feedstuffs are fair sources of it.

Thiamin

Little work has been done on the relation of thiamin to hatchability. Payne and Hughes (1933) reported that a deficiency of this vitamin reduces hatchability, but the fact that the diets used in their study were deficient in factors other than thiamin may have complicated the results. Ellis, Miller, Titus, and Byerly (1933) fed hens diets so inadequate in thiamin that many of the fowls showed polyneuritis, but some of the eggs hatched. The addition of good sources of thiamin did not greatly improve hatchability. When no thiamin supplements were fed the breeding hen, however, the chicks showed polyneuritis soon after hatching. Therefore, further work is desirable to demonstrate the role of thiamin in hatchability. The common cereal grains are good sources of this vitamin, and no deficiency is likely to occur among hens fed practical rations.

Other Vitamins

Inositol and para-amino benzoic acid have been shown to be essential for growing chicks under certain experimental conditions, but whether they act directly or stimulate intestinal synthesis of unknown factors is not known. The role of these factors in embryonic development has not been studied. The inositol content of eggs during incubation has been studied by Woolley (1942) and shown to remain constant, although the amount of free inositol does increase. The experiments of Rapoport (1940) showing that inositol is present as phytic acid in large amounts in chicken erythrocytes suggest that inositol may be important in embryonic development.

Aseorbic acid or vitamin C also has been shown to be synthesized during embryonic development (Ray, 1934; Suomalainen, 1939). The synthesis of vitamin C by the growing chick was

Pteroylglutamic Acid (Folic Acid)

Stokstad and Manning (1938) reported that growing chicks required an unknown factor, termed factor U, which was found in large amounts in alfalfa, middlings, wheat bran, and yeast. Snell and Peterson (1940) reported that in the norite eluate of yeast extracts there was present a factor essential for the micro-organism, *Lactobacillus casei*. This was termed the "norite eluate factor." Hutchings, Bohonos, Hegsted, Elvehjem, and Peterson (1941) indicated that the same factor was required for chicks. Other unidentified factors that were being studied at about the same time by other workers were termed vitamin B_c, folic acid, and factor R (Pfiffner and Hogan, 1946). A relationship among these various factors was apparent when synthetic pteroylglutamic acid became available and was found active in various experimental diets. The existence of conjugated forms of this vitamin, which had different activities for the test micro-organisms, resulted in considerable confusion among various workers until the pure vitamin became available.

Cravens, Sebesta, Halpin, and Hart (1942b) demonstrated that the "eluate fraction" of solubilized liver extract contained a factor or factors essential for the reproduction of chickens. Bauernfeind and Norris (1939) and Schumacher, Heuser, and Norris (1940) also encountered a deficiency of this vitamin in their studies with pantothenic acid in the nutrition of the mature fowl. The unidentified factor was termed factor R. Since then it has been suggested that the factor R preparation was a mixture of folic acid conjugates (Charkey, Daniel, Farmer, Norris, and Heuser, 1947).

Recently Taylor (1947) demonstrated that folic acid is essential for hatchability. Considerably less of the vitamin was required for egg production than for hatchability, although no definite requirements were suggested by Taylor. Embryos from folic-acid-deficient hens showed no characteristic gross pathological symptoms. The greatest increase in embryonic deaths occurred subsequent to the seventeenth day of incubation. Marked differences in the quality of the chicks hatched were observed; the poorer hatches from the diets low in folic acid were characterized by slower finishing of the hatching process as well

experimental groups deficient in calcium, but these authors did not believe this to be the primary cause of death of the embryos.

Studies by Titus, Byerly, Ellis, and Nestler (1937) and by others indicate that excessive amounts of calcium in the diet may cause a reduction in hatchability. Embryonic mortality was found to increase during the last 3 days of incubation. The detrimental effect of high levels of calcium was more pronounced with 0.9 per cent of phosphorus than it was with 1.2 per cent. Numerous studies have indicated that the efficiency of utilization of calcium and phosphorus is greatest when these minerals are present in the ration in certain ratios and that the ratio is influenced by the level of vitamin D fed. However, excessive minerals in the ration have been shown to decrease the efficiency of manganese utilization. The destructive effect of the minerals on certain vitamins has already been discussed. Free-choice feeding of the calcium supplement to breeding hens seems, therefore, the most satisfactory method of supplying this mineral. It also allows for differences in needs of individual birds arising from variations in rate of production and in size of egg.

The comparative value of the various forms of calcium for feeding laying hens has been the subject of many investigations. Heuser and Norris (1946) reported that a combination of oyster shell and granite grit was most satisfactory when egg production, egg-shell strength, body weights of the birds, feed utilization, and mortality were considered. This combination was compared with combinations of calcite grit and ground limestone with granite grit as well as with oyster shell, calcite grit, and ground limestone, singly. The detrimental effect on egg production of the feeding of dolomitic limestones has been demonstrated by several investigators (see Branon, 1938). The effect on hatchability of feeding dolomitic limestones to breeding hens seems worthy of study.

Apparently no studies have been conducted on the effect of phosphorus on hatchability. Studies by Miller and Bearse (1934), Norris, Heuser, Ringrose, and Wilgus (1934) and Evans, Carver, and Brant (1944) have indicated the level of phosphorus desirable for egg production, but unfortunately no hatchability data are presented. Hatchability studies on the effect of phosphorus would be of considerable interest and value, especially

demonstrated by Hart, Steenboek, Lepkovsky, Kletzien, and Halpin (1925) and by Carrick and Hauge (1925).

MINERALS

A comprehensive review of the minerals in poultry nutrition was published by Branon (1938). The chief studies of minerals in the nutrition of adult fowls have dealt with calcium, phosphorus, and manganese, perhaps because of the assumption, which may or may not be valid, that practical breeding rations carry adequate quantities of the so-called minor or trace elements. Research establishing the requirements of the breeding hen for the various trace elements and their effect on embryonic development would be of considerable value. Such studies are essential if the nutrition of the breeding flock is to be on a sound basis.

Studies of the role of minerals in hatchability are complicated by the interrelationships between the various elements and the indirect effects on hatchability due to egg-shell porosity. Under practical conditions, however, these effects are just as important as any possible direct effect on embryonic development.

Calcium and Phosphorus

Wheeler (1919) published a summary of data obtained over a period of many years which demonstrated the importance of calcium for laying hens. It was reported that egg production was lower and egg shells were thinner when the hen received inadequate supplies of calcium. Branon (1938) reviewed the many other studies dealing with calcium requirements and metabolism of chickens.

Buckner, Martin, and Peter (1925, 1929) demonstrated that calcium is essential for hatchability. In these studies hatchability decreased to zero when inadequate amounts of the element were supplied to the birds. The eggs and chicks hatched were smaller and contained less calcium. The authors state that fertility of eggs also decreased but, since the eggs were apparently not broken out and examined, it is possible that the early dead germs were not detected by candling. It was found that the evaporation during incubation was greatest in eggs from the

fore, be used to describe embryonic symptoms similar to those described above until histological examinations are made and the pathological picture is found to be identical with the classical concept of chondrodystrophy. Histologically, nutritional micro-

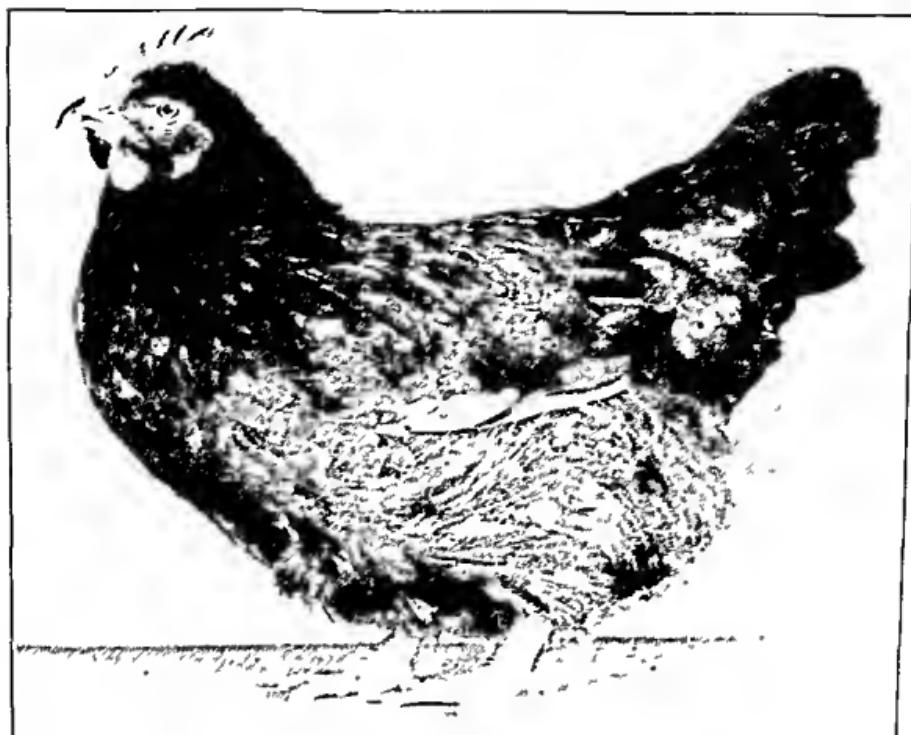


FIG. 10. Micromelia in adult fowl as a result of manganese deficiency in breeding ration of the dam. (From Caskey and Norris, 1940.) Courtesy Society for Experimental Biology and Medicine.

melia is readily distinguished from chondrodystrophy (Landauer, 1936).

A manganese deficiency in the diet of the hen has also been reported to cause a form of micromelia and a chronic congenital ataxia in day-old chicks which persisted in some chicks even to maturity (Caskey and Norris, 1940; Caskey, Norris, and Heuser, 1944). A mature bird exhibiting micromelia, as a result of a maternal manganese deficiency, is shown in figure 10. As mentioned previously, Couch and Cravens (1947) have found that chicks hatched from hens deficient in biotin exhibit a somewhat similar ataxic condition. Perhaps this should be expected in view

in view of recent work indicating that the utilization of cereal phosphorus varies according to the type of vitamin D used (see section on vitamin D).

Manganese

Shortly after it was reported by Wilgus, Norris, and Heuser (1937) that manganese is a factor in the prevention of perosis,

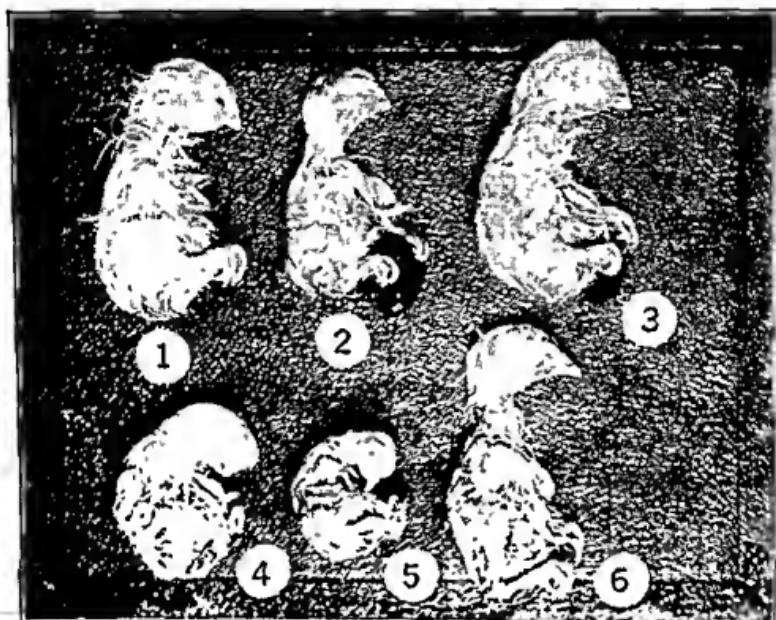


FIG. 9. Gross symptoms of embryos from manganese-deficient hens. Note generalized shortening of extremities. (From Insko and Lyons, 1937.)

Courtesy Kentucky Agr. Expt. Sta.

Lyons and Insko (1937) found that manganese is essential for hatchability. They observed a peak of embryonic mortality on the twentieth and the twenty-first days of incubation, and they reported also that the embryos showed characteristic symptoms which they termed chondrodystrophy. The embryos from eggs deficient in manganese exhibit short legs and wings, bulged head, parrot beak, and straight tibiae. Embryos with these symptoms are shown in figure 9. A similar condition of unknown cause, termed micromelia, had been described previously by Byerly, Titus, Ellis, and Landauer (1935) and studied histologically by Landauer (1936). The term nutritional micromelia should, there-

eggs by supplemental feeding have been unsuccessful. As mentioned previously, however, Erikson, Boyden, Martin, and Insko (1933) found an increase in the iron and copper content of eggs when the laying birds were exposed to sunlight.

Magnesium and potassium have been shown to be essential for growing chicks, but these minerals have not been studied in connection with breeding birds. Likewise no studies have been conducted with zinc, cobalt, and the other trace elements.

Selenium has been shown to be present in sufficient concentrations in feedstuffs grown in certain localities to cause low hatchability (Poley, Moxon, and Franke, 1937). Deformed embryos and monsters were found in a large number of the eggs failing to hatch. Edema and wiry down were also characteristic of selenium poisoning. Poley and Moxon (1938) showed that approximately 5 parts per million of selenium in the breeding hen's ration is the maximum amount that can be tolerated without producing a reduction in hatchability and a high chick mortality.

Fluorine has also been shown to be toxic for growing chicks, but there is no evidence that fluorine is detrimental to hatchability. Halpin and Lamb (1932) reported that egg production was slightly reduced whereas hatchability was not affected by feeding hens 3 per cent of rock phosphate containing 3.52 per cent of fluorine. Sources of phosphorus that are relatively low in fluorine are advisable, however.

Insoluble grit in the diet of hens has been the subject of several studies (see Branon, 1938). Although some investigators have reported no benefit from the feeding of insoluble grit to chicks, others find increased efficiency of digestion, especially of the whole grains. Even though grit may not be absolutely essential, it may be a wise procedure to allow birds to have free access to it.

The feeding of adsorbing charcoal has been shown by Ahnquist and Zander (1940) to prevent the utilization of part of vitamins-A, K, and riboflavin in the chick ration. In rations for breeding hens it appears undesirable.

of the relation of these nutritive factors in the prevention of perosis.

Numerous other studies have been reported on the role of manganese and its relation to other minerals in the diet of mature birds. Lyons (1939) found that the manganese content of the hen's diet affected the porosity and breaking strength of egg shells. Schaible and Bandemer (1942) and others working with chicks have demonstrated that excessive minerals in the ration make manganese unavailable and thus decrease its absorption from the intestinal tract. As mentioned previously, Couch, James, and Sherwood (1947) have suggested a possible supplementary relationship between vitamin D and manganese in the diet of breeding birds when a suboptimal level of vitamin D and an excess level of manganese is fed.

Other Minerals

Although it is generally accepted that breeding rations should contain 0.5 to 1.0 per cent of salt, no known studies have been conducted on the relation of salt or sodium and chlorine to hatchability. Prentice (1933) found that the feeding of rations deficient in salt resulted in a decrease in egg production and egg size, in a loss of weight, and in an increase in cannibalism. Excessive amounts of salt are toxic to chickens but the margin of safety is rather wide since the chickens can tolerate considerable salt.

The occurrence of goiter, caused by an iodine deficiency, has been reported by Welch (1928). Experimental congenital goiter also has been produced in chicks (Gassner and Wilgus, 1940). Numerous experiments have indicated that the addition of iodine supplements to practical breeding rations does not increase hatchability (see Branon, 1938); on the other hand, some reports from foreign countries do indicate that increased egg production and hatchability result from iodine supplementation. Wilder, Bethke, and Record (1933) and others have found that the iodine content of eggs varies depending on the iodine content of the hen's diet.

Though iron and copper are recognized as essential for hemoglobin formation in chicks, no studies appear to have been conducted on the role of these elements in egg production and hatchability. Attempts to increase the iron and copper content of

In addition to the importance of the unidentified factors in hatchability, it has been shown that there is a marked carry-over from the diet of the breeding hen to the chick (Bird, Rubin, Whitson, and Haynes, 1946; Wilgus and Zander, 1944; Bethke, Pensack, and Kennard, 1947). The symptoms that have been observed in chicks hatched from deficient hens are general weakness, failure to grow, high mortality, and perosis. The addition of fish meal, fish solubles, or cow manure to the ration of the breeding hen results in normal, vigorous chicks.

Not all investigators, however, have reported poor hatchability when rations containing soybean oil meal as the sole high-protein supplement were fed to breeding hens. Heuser and Norris (1944) found no difference in hatchability or egg production between hens fed rations high in soybean and those receiving soybean oil meal in combination with the animal-protein feedstuffs. Christiansen, Halpin, and Hart (1940) reported that the addition of manganese and riboflavin to rations containing soybean oil meal as the source of protein resulted in satisfactory hatchability. The addition of these materials was of special value in eliminating the slump in hatchability during the winter months which had frequently been associated with such rations. Bird and Marvel (1943) have shown that synthesis of riboflavin in the droppings and the ensuing coprophagy might explain the seasonal trends in hatchability. Rubin, Bird, and Rothchild (1946) have shown that a growth factor required by chicks fed rations high in soybean oil meal is present in the feces of hens. It thus seems that ingestion of feces may be responsible in part for the seasonal trends in hatchability observed when the hens are fed rations containing soybean oil meal as the sole supplementary protein. At the present time there is some controversy whether the need for the factor(s) present in certain products of animal origin is or is not a peculiarity of rations containing high levels of soybean oil meal. Since the need for such a factor or factors has been observed with other types of rations, it seems that soybean oil meal is not essential for the production of the deficiency. It is apparent, however, that at the present time it would be unsafe to rely on vegetable-protein feedstuffs as the sole supplementary source of proteins and unknown factors in breeding rations. Unfortunately the exact quantities of fish meal, liver meal, meat scrap, green

UNIDENTIFIED FACTORS ("ANIMAL-PROTEIN FACTOR," "COW-MANURE FACTOR")

As may be seen from table 2, the qualitative and quantitative needs of breeding chickens for a number of nutritive factors have been determined; the needs of breeding turkeys, however, have not been studied to any great extent. There is ample evidence that unidentified factors of considerable practical importance in breeding rations remain to be isolated and identified and the quantitative needs of the breeders for such factors determined. The distribution and stability of these factors in feedstuffs must also be determined before the results can be satisfactorily applied to practical rations.

Byerly, Titus, and Ellis (1933) observed that breeding rations containing soybean oil meal or cottonseed meal as the only source of supplemental protein would not support good hatchability. When such rations were supplemented with certain protein feedstuffs of animal origin, improved hatchability resulted. Nestler, Byerly, Ellis, and Titus (1936) distinguished this factor (or factors) from riboflavin and reported its presence in green grass, dried pork liver, and in a combination of meat meal, fish meal, and dried buttermilk. Although it is probable that the preceding experiments were complicated by a manganese deficiency, further evidence for the existence of such a factor (or factors) essential for breeding hens has also been reported by Hunt, Winter, and Bethke (1939), Wilgus and Gassner (1941), McGinnis, Heuser, and Norris (1944), Cravens, Halpin, and McGibbon (1946), and by others.

Somewhat variable results have been reported by the various investigators working on the problem, especially in regard to the distribution of the factor or factors. This is not surprising in view of the different types of test diets employed and the variation in samples of ingredients tested as possible sources of the factor. From the evidence available at the present time, it appears that the best sources of the unknown factors are fish meal and fish solubles, liver meal, certain liver fractions, and cow manure. Meat seraps, milk products, and green feeds appear to contain somewhat smaller amounts of the factor(s).

A deficiency of manganese in the diet of rabbits has been shown to result in testicular degeneration in the male (Smith, Medlicott, and Ellis, 1944). Whether male chickens and turkeys would react in the same manner is problematical.

The effect of feed restriction on fertility in males has been studied by Parker and McSpadden (1943), and a pronounced decrease in fertility and semen volume was observed with inanition. Such results emphasize the importance of the use of feed hoppers so designed that the male can eat freely.

Some reports on hatchability have indicated that fertility may have been reduced by the ration fed. However, in view of the work of Munro and Kosin (1945) showing that many dead germs may be mistaken for infertile blastodiscs, it seems likely that a more detailed examination of the eggs in order to separate early dead germs from infertile eggs will be necessary for the accurate study of fertility as well as hatchability. Studies on the nutrition of the male appear to be a worthwhile field for research.

RECOMMENDED NUTRIENT ALLOWANCES

The recommended nutrient allowances for breeding hens among chickens and turkeys are given in table 2. These allowances represent the best interpretation of studies on the nutritive requirements of poultry and their evaluation for practical feeding conditions that could be made by a committee of poultry nutritionists of the National Research Council (1946). The allowances given are based on optimum requirements as determined experimentally, plus an added margin of safety to allow for breed and strain variations, variations in nutrient content of feedstuffs, and some loss of nutrients during a reasonable period of storage. It may be pointed out that the vitamin D allowance for breeding turkeys may not appear to be in accord with published experimental work on the subject. This apparent disagreement arises from the work of Bird (1944) and Boucher (1944) who showed that the growing poult does not utilize the vitamin D from fish oils as efficiently as that from irradiated animal sterols. The allowance for growing poult was first determined, and then that of the turkey breeding hen was set at the same figure because it was believed that the hen would not require less than the growing

feeds, and the milk products that should be present in a breeding ration to supply the unknown factors cannot be stated because of the variation in the quality of such materials. It is also probable that other materials will be found of value in supplying the unknown factors. Such materials as yeast from different sources, fermentation products, and residues should be more thoroughly studied in this connection.

NUTRITION AND FERTILITY

The relation between nutrition and fertility has received very little attention by poultry nutritionists. Hatano (1935) compared the effect of diets composed of vegetable proteins with those composed of animal proteins on number of copulations, duration of fertility, and number of fertile eggs produced by the hen in a single mating. A slight reduction in number of copulations and in fertile eggs from a single mating was found for fowls fed the diet of vegetable proteins, but the differences were small and, if significant, probably could not be attributed to the protein of the diet. Craft, McElroy, and Penquite (1926) presented data indicating that males fed rations low in vitamin A produced fewer sperms than males receiving adequate vitamin A, but the data were insufficient to determine whether or not fertility was affected to any significant degree.

Adamstone and Card (1934) fed a ration treated with ethereal ferrie chloride for the destruction of vitamin E to male chickens for 1 year and found that the fowl produced sperm still capable of fertilizing eggs. After 2 years on such a diet some of the males were sterile. Histological study revealed that the testes of 1 male were completely atrophied while the remaining 3 males showed definite degenerative changes. Abnormal sperms were observed very shortly after the experiment was initiated. These authors believed that the testis is very resistant to vitamin E deficiency whereas spermatogenesis is quickly affected. It should be emphasized, however, that more recent experiments have shown that ferrie chloride treatment does not completely destroy the vitamin E in a practical diet. Titus and Burrows (1940) found that the addition of 0.5 per cent of cold-pressed wheat-germ oil to the diet of males caused a marked decrease in semen production.

Feed Consumption

Besides supplying all the nutritional essentials, a good ration must also be eaten readily and be relished by the bird. Such a ration is often said to be palatable. Although the term palatability has a somewhat vague meaning, it is well recognized by poultrymen that some feeds are more readily consumed than others. The texture of the mash may be quite important since birds do not relish dusty or powdery mashes. Moldy or musty feeds should never be supplied the breeding flock. Such feeds may cause a reduction in mash consumption and also a reduction in hatchability since the mash is the chief carrier of many of the nutritive factors so essential for good hatchability.

Some fiber in poultry rations seems to be desirable, but excessive amounts should be avoided because of the limited capacity of birds to utilize it and the need of the breeding birds for concentrated feeds. Approximately 5 per cent seems to be a satisfactory amount (Heuser, 1946). It is recognized, however, that excellent results have been obtained with rations containing much more or much less than this amount of fiber. It is of considerable interest in this connection that Davis and Briggs (1947) have recently reported that the addition of fiber to a "synthetic" diet caused a stimulation in growth of chicks. Obviously further studies on the amount of fiber in poultry rations are warranted.

No one system of feeding breeding birds is recommended. Probably the most commonly used system or method of feeding is the mash and grain system, but it is well recognized that satisfactory results may be obtained by other methods. In feeding for hatchability a system that insures a liberal intake of the feedstuffs that are rich in those minerals and vitamins most likely to be deficient in a breeding ration is the important consideration.

It should be remembered that in the practical feeding of poultry we are observing the reactions of the flock, and not the individual bird, to a feed or system of feeding. Individual birds vary markedly in the ratio in which they consume mash and grain as well as in their choice of individual grains. Feed consumption may also vary with the season of the year and the climate. Studies on the nutritive needs of individual birds and the effect

TABLE 2

RECOMMENDED NUTRIENT ALLOWANCES FOR CHICKEN AND
TURKEY BREEDING HENS

	Amount of nutrient per pound of feed or per cent	
	Breeding chickens	Breeding turkeys
Total protein (per cent)	15	15
Vitamins		
vitamin A activity (I.U.) *	3300.0	4000
vitamin D (A.O.A.C. units)	450.0	800
thiamin (mg.)	?	?
riboflavin (mg.)	1.3	1.8
pantothenic acid (mg.)	5.0	?
nicotinic acid (mg.)	?	?
pyridoxin (mg.)	1.6	?
biotin (mg.)	0.07	?
choline (mg.)	?	?
pteroylglutamic acid (mg.)	?	?
vitamin K (mg.)	?	?
Minerals		
calcium (per cent) †	2.25	2.25
phosphorus (per cent) ‡	0.75	0.75
manganese (mg.)	15.0	15.0
salt (per cent) §	0.5	0.5
iodine (mg.)	0.5	?

* May be fish-oil vitamin A or provitamin A from vegetable sources.

† This amount of calcium need not be incorporated in the mixed feed inasmuch as calcium supplements fed free choice are considered as part of the ration.

‡ Inorganic phosphorus should constitute 0.2 and 0.4 per cent of the total feed for chickens and turkeys respectively.

§ This figure represents added salt or sodium chloride.

poult. Obviously, this allowance, in particular, is subject to modification as further data become available on the subject.

FEEDING PRACTICES

Breeding rations must be designed to supply all the nutritive essentials in the proper amounts and in addition must meet certain other requirements if satisfactory results are to be obtained.

SOME PRACTICAL APPLICATIONS

In this review, basic nutritional findings have been considered from the standpoint of their application to feeding breeding hens. It has been pointed out that the diet fed the breeding bird is of extreme importance in determining the number and vitality of the chicks and poult's that hatch. This carry-over may have a measurable beneficial effect on the offspring for several weeks of its life.

The effect of a particular nutritive deficiency in the diet of the hen on age at death and gross pathological symptoms of the embryo has been considered, but unfortunately data are lacking for many of the nutritive factors. If such data were obtained for the nutritive essentials unstudied in this regard, it might be possible to detect these deficiencies in the diet of the hen by an examination of the embryos dying in the eggs. Histopathological studies of embryos dying as a result of a specific deficiency might also be of considerable value in this respect.

The need for certain other types of research has been mentioned. This need is particularly apparent for turkeys and for breeding males. In addition many basic nutritional problems remain to be studied. Professor E. B. Hart (1937) of the University of Wisconsin has stated, "We should chart all the factors in nutrition, organic and inorganic, and study their distribution, physiology, pathology and interplay. Put the need for these factors on a quantitative basis with optimum allowance for the complete cycle of the animal's life." Professor C. A. Elvehjem (1946) has also discussed future studies in nutrition in an interesting and stimulating manner.

In designing rations that supply the known nutritive allowances plus the unknown factors, emphasis has been placed on a combination of feedstuffs of high quality. The available information indicates that liberal quantities of some combination of marine products, meat scraps, green feeds, milk products, certain fermentation products, and residues are desirable in a good breeding ration. A combination of these feeds with the cereal products and vegetable-protein feedstuffs, adequately supplemented with the minerals and fat-soluble vitamins, can be relied on to nourish

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the breeding flock adequately. There seems to be no valid reason for placing the burden of supplying essential nutritive factors on any one feedstuff.

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CHAPTER 2

Formation of the Hen's Egg

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The egg constitutes the bridge between succeeding generations of chickens. The fertilized egg not only carries the hereditary units that determine the make-up of the chick to be hatched but

also contains the nutrient materials that support the chick during its entire embryonic development. The hereditary aspects are determined early by a chance combination of available genes, but the welfare of the developing chick is largely dependent upon the deposition of the nutrients and the protective envelopes of the egg. Thus the process of egg formation has a vital influence on the survival and the quality of the resulting chick.

STRUCTURE OF THE FEMALE REPRODUCTIVE ORGANS

The reproductive organs of the female chicken are unusual in that they are asymmetrical. In most vertebrates the ovaries and

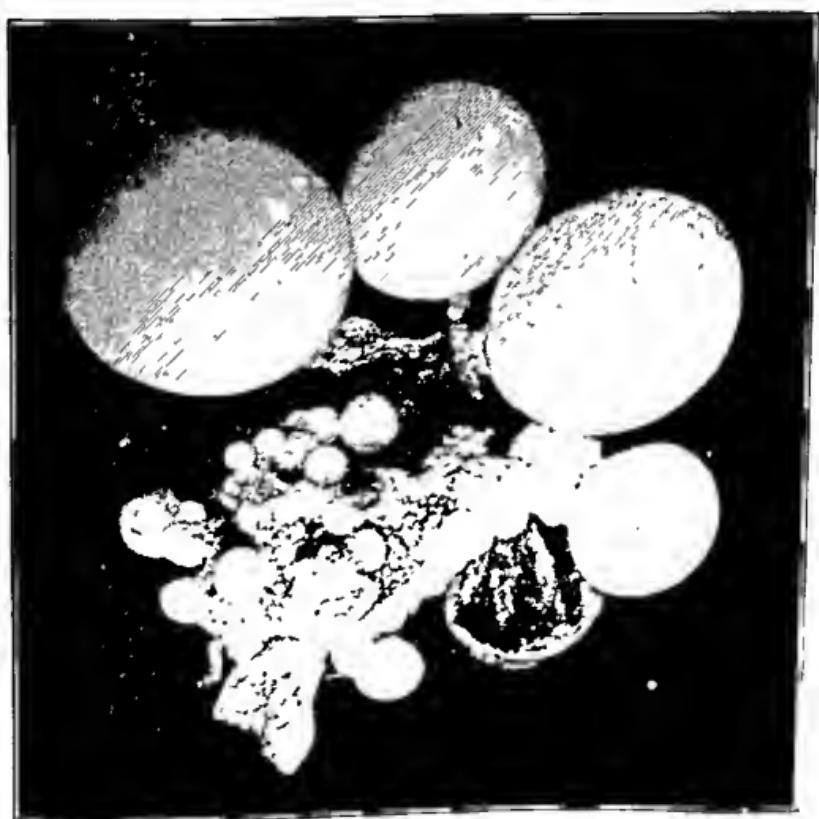


FIG. 11. Ovary of a laying hen showing four rapidly growing ova. The dark-appearing ruptured follicle had released its ovum only a few hours before; another partially resorbed ruptured follicle is also seen.

Formation of the Hen's Egg

oviducts are paired, but in the chicken the right ovary and oviduct cease development in the early embryonic stages. Thus

there is normally only one functional ovary and oviduct in the hen. In the laying hen, the ovary is seen as a cluster of developing ova (yolks) varying from very small to full size (fig. 11). There are usually not more than a half dozen of the larger ones which are yellow, whereas the small white ones number in the hundreds. Pearl and Schoppe (1921) found 900 to 3600 ova in laying females but observed no relationship between the number of visible ova and the egg-production capacity of individual birds. The functional ovary shows ruptured follicles in varying stages of resorption. These are the remains of the membranes which enclosed the yolks (ova) and attached them to the ovary.

The oviduct is a tube having many loops through which the yolk passes for fertilization and the addition of the white and surrounding membranes. This tube extends from its attachment at the base of the ovary to the cloaca. The oviduct varies in structure throughout its length in accord with its varying functions, the different portions of this duct being specialized for the secretion of the different parts of the egg. The oviduct has the following five major parts: (1) the infundibulum or funnel which picks up the yolk after release from the ovary, (2) the magnum where most of the egg white is secreted, (3) the narrowed isthmus where the shell membranes are formed, (4) the pouch-like

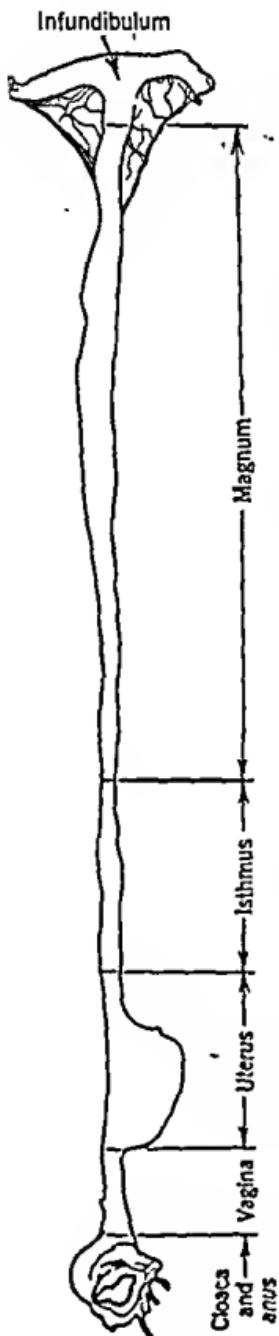


FIG. 12. A diagrammatic sketch of a hen's oviduct showing the location of its parts.

uterus in which additions to the white are made and the shell is deposited, and (5) the vagina which leads from the uterus to the cloaca from which the egg is expelled (fig. 12). There is no sharp line of demarcation between most of these regions of the approximately 22-inch oviduct, of which the magnum comprises over 50 per cent and the isthmus and uterus each about 15 per cent. The size of the oviduct is much reduced when a female is not in laying condition.

STRUCTURE OF THE EGG

With the exception of the shell, white, and yolk, a casual observer would assume that the egg has no special structures; closer examination, however, reveals a complex make-up. The shell of the egg is lined with two membranes which adhere to each other closely, except at the large end where the air cell occupies a space between the two. If a cross section could be made of the white, it would be found to be divided into three distinct areas; a layer of thick viscous white occupies the median area between two layers of watery white, one of which is adjacent to the yolk and the other next to the shell (fig. 13). A fourth structure of the white, the chalazae, consists of firmly twisted strands of fibers attached to the yolk at opposite sides and extending in the direction of the long axis of the egg. The chalazae have been supposed to act as a stabilizer of the yolk, although the evidence favoring this idea is not conclusive. The mechanism of the formation of the chalazae will be discussed later.

The egg yolk (or ovum) is enclosed in the noncellular vitelline membrane. The structure is best demonstrated by slicing a hard-boiled yolk through the blastodisc (germ spot). The blastoderm, which is seen at the center of the yolk, is less readily congealed by heat than is the surrounding portion of the yolk and is largely composed of that part of the ovum existing at the stage just previous to the period of rapid growth. A narrow neck of similar material leads from the central core to the blastodisc. Many illustrations of the hen's ovum show alternating concentric bands of light and dark yolk. Such bands may be seen in some hard-boiled egg yolks but are missing in many others. Conrad and Warren (1939) have shown that the presence or absence of bands

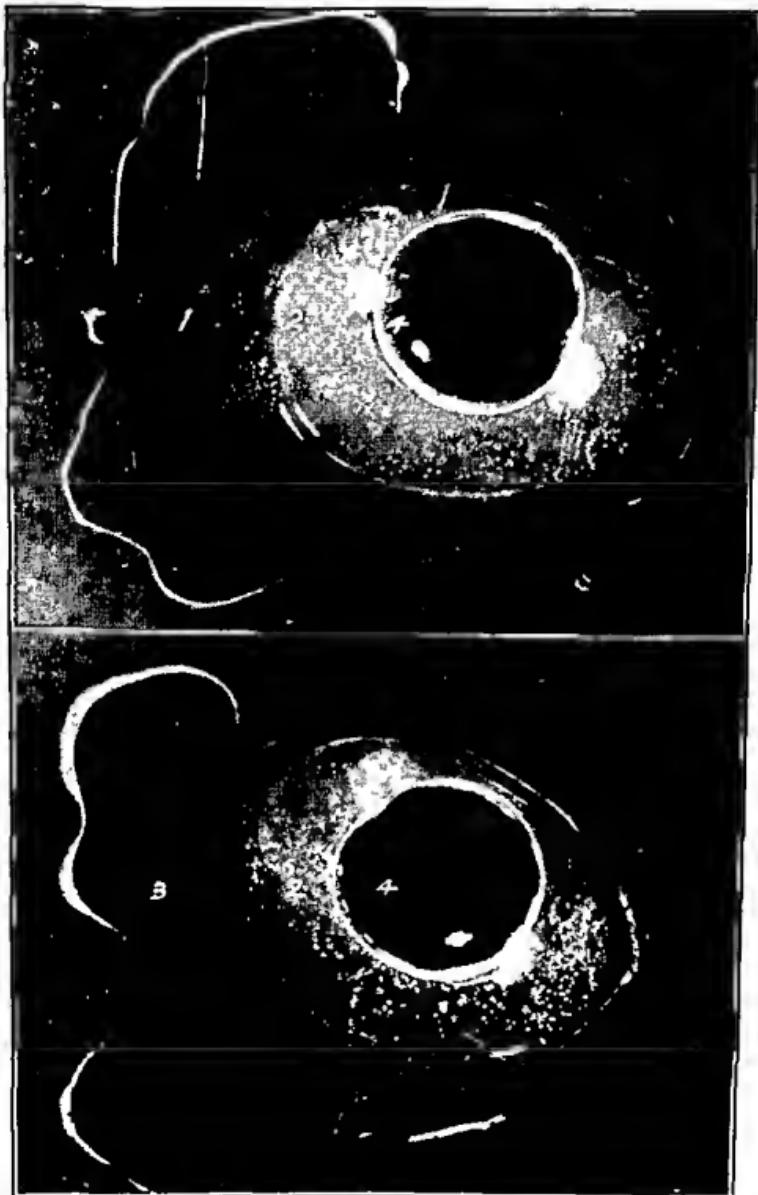


FIG. 13. Broken-out eggs showing the various parts of the white. The upper view shows the outer thin white (1), the thick viscous layer (2), and the yolk (4). The lower view shows an egg with the outer thin white removed and the viscous portion cut so as to release the inner thin white (3). The chalazae, unlabeled, can be seen as whitish twisted cords on opposite sides of the yolk in each of the eggs.

in the yolk is dependent upon feeding practices. The color of the yolk is influenced by the type of food consumed, the yellow color being determined largely by the xanthophyll content of the feed. Bohren, Thompson, and Carriek (1945) found that, when the diet of laying hens was changed from a feed high in carotenoid pigments to one low in these pigments, some color was deposited in the yolk for a considerable period after the change had been made, a fact that indicated that pigment may come from the body tissues as well as from the feed. Other pigments in the feed are known to affect the color of the yolk.

Stewart (1935) has presented an excellent review of the literature on the structure of the egg shell. Since the shell both protects the developing embryo and permits gaseous exchange with its external environment, its structure is of interest in connection with problems of incubation. The parts of the shell are the inner mammillary layer, the spongy layer, the cuticle, and the pores. The spongy layer comprises approximately two-thirds of the entire shell thickness. The outer covering of the shell, known as the cuticle or bloom, is popularly believed to function in reducing evaporation; however, Bryant and Sharp (1934) found that the removal of the cuticle by washing did not increase evaporation. Marshall and Cruickshank (1938) suggested that the cuticular plaques accelerated rather than retarded evaporation. The pores in the shell, which range in number from 6000 to 8000, originate in the mammillary layer and open to the outer surface through countersunk areas on the surface of the egg.

GROWTH OF THE OVUM

The earliest stages of egg formation take place in the ovary and involve the growth of the ovum or yolk. Observation of a hen's functional ovary shows a large number of follicles containing ova of gradually increasing size, only a few of which approach mature size, and this seems to indicate that the period of rapid growth is of relatively short duration. The early stage of development of the ovum is so slow that it is difficult to obtain an accurate measure of rate of growth (Marza and Marza, 1935), but the later, more rapid growth has been fairly accurately recorded. One method of study was to kill females of a known rate of laying

OVULATION

Ovulation is the process of release of the yolk or ovum from the ovarian follicle. Details of the process of ovulation were first reported by Warren and Scott (1934), and the mechanics of ovulation were further studied by Phillips and Warren (1937).



FIG. 15. A nearly mature follicle showing the network of blood vessels and the clear area known as the stigma along which rupture occurs to release the ovum.

A mature follicle shows a nonvascular streak known as the stigma (fig. 15), and it is along this streak that the rupture occurs for release of the ovum. The blood vessels radiate out from the stalk attaching the follicle to the ovary but terminate before reaching those from the opposite side, thus leaving the clear area, the stigma. The blood vessels of the follicular membrane become quite prominent with approaching maturity of the enclosed ovum. In the last hour before ovulation the blood vessels become somewhat blurred, and this blurring is the basis for identifying impending ovulation. At this time, in addition to the blurring of the follicular blood vessels, there is a distortion of the stigma. The fact that application of pressure on the fol-

lieular membranes of less mature ova has a similar effect on the blood vessels suggests that increased internal pressure is a factor in ovulation. If ovulation is not too rapid, it can be seen that, in some instances, the inner layer of the follicular membrane ruptures first and thus causes a bulging of the outer layer. Once the break is complete, the release is virtually instantaneous. Usually the rupture starts at one end of the stigma and continues throughout its length.

Numerous hypotheses have been proposed to explain the mechanics of ovulation. In the work of Phillips and Warren previously mentioned, a critical study was made of some of these theories. No support could be found for the idea that fluids accumulating under the follicular membrane were responsible for its rupture. There was also no evidence for any last-minute surge in yolk deposition which could cause ovulation. It had been suggested (Patterson, 1910) that grasping of the follicle by the funnel of the oviduct could be responsible for the rupture of the surrounding membrane, but observations of Phillips and Warren did not support this view. Histological examination of follicular membranes failed to provide any support for the theory that the release of the ovum might be due to enzymatic action weakening the follicular membrane. It was found that neither clamping off the follicular stalk a few minutes before ovulation nor the complete excision of the follicle from the ovary an hour before ovulation prevented the release of the ovum. Hence ovulation seems not to be dependent upon any stimulus coming from the body of the female at the moment of ovulation. Phillips and Warren suggested that ovulation was the result of a prolonged tension of the muscle fibers of the follicular membrane. The factors causing the tension were not identified.

The stimulus that starts the mechanism responsible for ovulation has in recent years been given active study. Fraps and co-workers in the Bureau of Animal Industry in the United States Department of Agriculture have made significant contributions in this field of study. Fraps and Riley (1942) found that ovulation could be directly effected by injection of an anterior pituitary preparation rich in luteinizing hormone. If this treatment took place after 6 to 11 days of pretreatment with pregnant-mare

serum, which causes growth of many follicles but at the same time suppresses ovulation, multiple ovulations involving as many as seven follicles could be induced. Nalbandov and Card (1946) confirmed these findings. Fraps, Olsen, and Neher (1942) found that ovulations could be induced prematurely by as much as 17 hours in the regularly laying hen. Fraps and Dury (1942) later reported the first follicle of a clutch sequence to be much more sensitive to intravenously injected, ovulation-inducing preparations than subsequent follicles.

Rothechild (1946) has demonstrated, by means of hypophysectomy, that the release of ovulation-inducing hormone from the hen's anterior pituitary gland takes place some 4 to 5 hours before ovulation. This interval is in agreement with the period of 6 to 8 hours elapsing between intravenous injection of ovulation-inducing preparations and consequent ovulation (Fraps, Riley, and Olsen, 1942). Fraps and Dury (1943) reported luteinizing fractions of anterior pituitary origin (from sheep) to be much more effective than any other gonadotrophin in the induction of ovulation in the hen, as is also generally true for mammals. More recently, a presumptive luteinizing fraction from the anterior pituitary glands of male chickens has proved effective at a level of 0.0005 to 0.001 milligram (Fraps by correspondence). If these various findings are taken together, there seems little doubt that the ovulatory process in the hen is under the immediate control of a hormone, probably luteinizing in character, elaborated and released by the anterior pituitary gland. The mechanism effecting release of the hormone remains obscure.

As is well known, females tend to maintain a characteristic interval between the successive eggs of a clutch, the females with the larger clutches having the shorter intervals. The interval is usually longer than 24 hours. Thus a female with a 28-hour interval lays at 8 A.M. today, 12 noon tomorrow, and 4 P.M. the next day. If laid on schedule, the next egg would come at 8 P.M., but hens usually do not lay so late in the day. The normal procedure is for the bird to miss a day and then start a new clutch of eggs at approximately 8 A.M. on the day following. At one time it was believed that the 8 A.M. egg of the new clutch was held in the shell gland overnight and released in the morn-

ing. However, Scott and Warren (1936) showed that the break in the sequence in laying was due to a delay in ovulation and not to retarded egg formation and that the first egg of a clutch is in the oviduct a period of time similar to that of succeeding eggs.

Warren and Scott (1936), through the use of continuous lighting and reversed periods of daylight and darkness, were able to demonstrate that the onset of darkness was a factor influencing the termination of the clutch and the consequent restriction of egg laying to the daylight period. When the periods of light and darkness were artificially reversed, birds could be caused to lay all their eggs during the night hours, but there was about a 60-hour lag in the onset of the change. These experiments indicated that the regulating effect of light antedated ovulation. Fraps, Neher, and Rothchild (1947) placed birds under continuous lighting but failed to get them to lay over the entire 24-hour day as did Warren and Scott, as well as McNally (1946). Fraps and co-workers conclude that photoperiodicity is not a necessary limiting factor in regulating time of lay but that oviposition may be regulated primarily by factors which determine bodily activity.

Ovulation was found to succeed laying of the egg (oviposition) by an average interval of 32 minutes (Phillips and Warren, 1937). This short interval was recorded for eggs laid on successive days and *may be considered as indicating that oviposition bears some causative relationship to ovulation*. However, the fact that some 14 or more hours elapse between the laying of the last egg of a clutch and the ovulation of the first egg of the succeeding clutch is evidence against this view. The accompanying photograph (fig. 16) showing two eggs in an oviduct is a further refutation. On the other hand, some evidence indicates that processes associated with ovulation may, within limits, control time of lay even though oviposition occurs before overt ovulation. Fraps (1942) reported that, when ovulation is forced some 3 to 6 hours before a uterine egg is normally due to be laid, the egg is laid in close association with the forced ovulation, i.e., 3 to 6 hours prematurely. Ovulation is readily forced at much greater intervals than 6 hours before oviposition of a uterine egg is due, but under these conditions oviposition does not occur prematurely.

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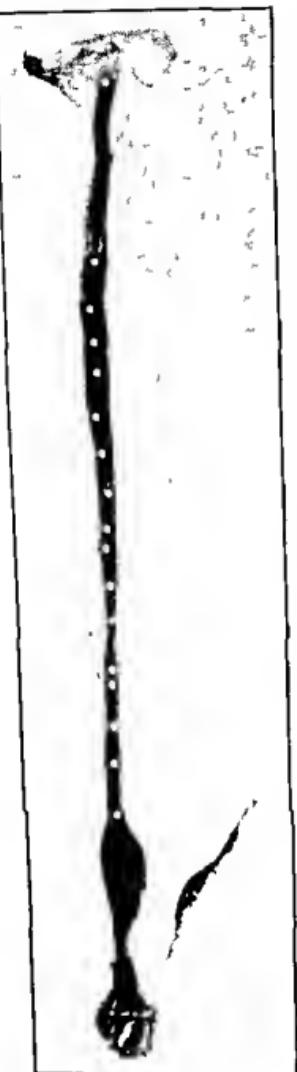
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mation of rate of egg movement. It was found that through the use of a proper anesthetic it was possible to observe directly the normal procedure in egg formation, including ovulation and the subsequent engulfment of the ovum by the infundibulum (Warren and Scott, 1934). At the time of ovulation, intense peristaltic activity is incited in the anterior portion of the oviduct. Movements of the infundibulum are at random, and contact with the released ovum seems to be due to chance. Contact, when once made, may be broken, and the actual engulfment may take from 5 to 25 minutes.

Propulsion of the Egg

The relative effectiveness of ciliary and peristaltic action in propelling the ovum through the oviduct is not known. Parker (1930) has shown that there are some ciliary tracts which beat in a posterior direction as well as those exerting an anteriorly directed force. The latter tracts are thought to function in transporting the spermatozoa through the oviduct. The fact that peristaltic action is much more intense just before and during the passage of the egg suggests that it also plays a part in transporting the egg. In the early stages of engulfment of the ovum, the obvious distortion of its shape suggests that peristalsis is playing a part in the propulsion of the ovum through the oviduct. Many cilia exert a force in the direction of the moving egg, but the evidence for the importance of ciliary action in the movements of the egg is not convincing. Although the constant and virtually imperceptible movement of the egg through the oviduct is the type expected from cili-

FIG. 17. The oviduct of a hen showing the time schedule of the forming egg. The small white discs on the oviduct mark the progress of the egg at 15-minute intervals. This bird is unusual in having a sizable right oviduct.



ary rather than from peristaltic force, the almost continuous serpentine contortions of the oviduct resulting from wave-like peristalsis while the egg is in passage cannot be ignored. It is possible that both peristaltic and ciliary action have their effect on the movement of the egg through the oviduct. The general shape of the egg, which is assumed even before the white is enclosed in the shell membranes, may bear some relation to the method of transportation. In the posterior magnum and in the isthmus the pointed end of the moving egg is the advanced part.

Rate of Passage Through the Oviduct

Warren and Scott (1935a) obtained fairly accurate timing of the passage of eggs through the oviducts of 5 hens. These birds were anesthetized immediately before ovulation, and markers were placed on the oviduct just posterior to the forming egg at 15-minute intervals (fig. 17). After oviposition, the oviducts were removed, and a mean time schedule was calculated for each region of the oviduct. The calculated time schedule was 18 minutes spent in the infundibulum, 2 hours and 54 minutes in the magnum, 1 hour and 14 minutes in the isthmus, and 20 hours and 40 minutes in the uterus and the vagina. This gave a 25.1-hour period which is relatively short; however, hens having relatively high laying rates were chosen for the experiment. Autopsies were performed on 40 hens, and the time schedule thus obtained for the progress of the egg through the various parts of the oviduct agreed well with the data obtained from observation of anesthetized hens. Warren and Scott (1935b) found that in low-rate layers most of the increased interval was accounted for by extra time spent by the egg in the uterus. Heywang (1938) found that hens laying in long clutches tended to have a slightly longer interval between the first two eggs and the last two eggs of a clutch.

ALBUMEN SECRETION AND DEPOSITION

In the Infundibulum

The observations of Warren and Scott (1935a) indicate that the ovum passes rather rapidly through the short infundibulum, and therefore but little albumen deposition can be expected. There is no sharp line of demarcation between the infundibulum

and the anterior magnum. The infundibulum usually has been defined as that portion of the oviduct anterior to where the secretory folds begin. Since the ovum is already entering the magnum by the time it is fully engulfed, the secretory action of the infundibulum may be considered as negligible.

In the Magnum

Numerous workers have studied the secretion of egg white in the magnum (albumen-secreting section) by various methods of approach. Those making microscopic observations were Coste (1847), Pearl and Curtis (1912), and Chomkovic (1927). Histological studies of the magnum and its secretions were made by Cushny (1902), Surface (1912), Bradley (1928), Richardson (1935), and Cole (1938). Operative techniques involving resection of the magnum were used by Asmundson (1931), Asmundson and Jervis (1933), Asmundson and Burmester (1936), Scott (1938), Burmester and Card (1939), and Burmester (1940).

Richardson (1935) suggested that the cephalic portion of the magnum, which he called the "chalaziferous region," was the section in which the chalazae of the egg were secreted. This view is not shared by other workers. Burmester and Card (1939) found that the resection of the chalaziferous region had no influence on chalaza formation. A more acceptable theory of the mechanics of the formation of the chalaza will be presented later.

The white of a freshly laid egg has five parts: the outside thin white, the thick white, the inside thin white, the chalaziferous layer, and the chalazae. An egg removed from the upper end of the magnum, however, shows none of these divisions. At that stage the albumen is a thick jelly-like mass with no differentiation. As the egg leaves the magnum to enter the isthmus, examination may show a small amount of the inside thin white adjacent to the yolk, and this differentiation of the inside thin white and the chalazae continues as the egg passes through the isthmus and into the uterus.

Pearl and Curtis (1912), McNally (1934), and Scott, Hughes, and Warren (1937) are agreed that approximately one-half the volume of the egg white is deposited when the egg leaves the magnum and that the concentration of proteins (per unit of

white) in the egg entering the isthmus is about twice that in a laid egg. Conrad and Scott (1942) found that proteins of the general character of egg white accumulate in the magnum at a fairly constant rate during the interval between the passages of eggs and even during the interval between clutches. Asmundson and Jervis (1933), Asmundson and Burmester (1936), and Scott (1938) found that resections of the magnum significantly reduced the amount of albumen in the egg. Asmundson and Burmester (1936) and Scott and Burmester (1939) found not only that removal of parts of the magnum tended to reduce the percentage of white in the egg but also that there were qualitative differences in the albumen according to the part of the magnum resected. Resection of the anterior portion of the magnum reduced the amount of the inner thin portion of the egg white, whereas operation on the posterior magnum tended rather to reduce the amount of the thick egg white. These results point to the probable specialization of different regions of the magnum in the type of albumen secreted.

In the Isthmus

Whether or not the isthmus contributes anything other than membranes to the egg has been a matter of controversy. The answer to the question has been sought by comparing the percentage of solids in the white of eggs when they are entering and when they are leaving the isthmus. About 95 per cent of these solids is protein. Pearl and Curtis (1912) reported some increase in the percentage of nitrogen in the egg white during its passage through the isthmus; thus an addition of protein to the fluids of the egg white while they are in the isthmus is indicated. Scott (1938), however, found no evidence for any additions to the white at this stage. Burmester (1940), from a careful comparison of the amounts of white before the egg entered and after it left the isthmus, stated that the evidence is quite conclusive that water is added to the egg as it passes through the isthmus; however, the observed increase in the weight of the white was not statistically significant. Asmundson (1939b) in the study of turkey eggs found support for the view that some water is added in the isthmus. It would appear that small additions to the white are made during the egg's passage through the isthmus.

In the Uterus

About 80 per cent of the time required for egg formation in the oviduct is spent in the uterus, or shell gland. Observation of the process of egg formation is less illuminating at this stage since little can be seen directly other than the constant peristalsis of the uterus. In order to learn what takes place in the uterus, attention has been focused on the changes taking place in the egg itself. The methods of study have been resection of the uterus, autopsies, palpation, and premature expulsion of the egg. Techniques have been worked out by means of which the egg can be expelled at any time after it reaches the uterus without injuring the female. This has permitted the establishment of an accurate time schedule of changes taking place in the uterine egg. Numerous workers have observed that the egg bears loose-fitting membranes upon arriving in the uterus and that the egg soon becomes plump by taking up uterine fluids. It also has become a well-established fact that not all the various layers—chalaziferous (including chalazae), inner thin white, thick white, outer thin white—into which the egg white is differentiated are laid down in specific portions of the oviduct but are the result of changes taking place in the albuminous mass as it accumulates.

Pearl and Curtis (1912) found what they considered to be good evidence that protein (albumen) was added to the uterine egg. Hansen (1933) pointed out that it was improbable that protein in a dilute solution would be able to diffuse into the egg after the membranes were formed. However, McNally (1933, 1934) carried out studies in which he interpreted his data as supporting the findings of Pearl and Curtis. Scott, Hughes, and Warren (1937) reexamined the data of Pearl and Curtis and carried out further studies which seemed to demonstrate fairly conclusively that no protein was added to the egg-white mass after it was enclosed in its membranes. Their evidence was: First, the laws of physical chemistry make it impossible for uterine fluid with 0.4 per cent protein concentration to pass through the membranes into the white mass which contains approximately 20 per cent protein. Secondly, Pearl and Curtis, as well as McNally, in determining the relative protein concentration of isthmian eggs, always used the first egg of the clutch as the control or laid egg.

They found that this laid egg possessed a higher absolute amount of protein than the egg intercepted in the isthmus and concluded from this that protein was added after the egg membranes were formed. Scott, Hughes, and Warren found that the first egg of the clutch normally had a sufficiently greater amount of protein than succeeding eggs to account fully for the additional amount of protein which the other workers had attributed to secretions of the uterus. Thus it appears that protein does not pass through the egg membranes and that the protein content of the outer thin white, which is developed while the egg is in the uterus, comes from the adjacent mass of thick white. Asmundson (1939a) found that no protein was added to the turkey egg after it left the magnum.

Beadle, Conrad, and Scott (1938) found that the uterine fluid surrounding the membranous egg in the uterus was not albuminous, but a mineral solution consisting chiefly of sodium, calcium, and potassium existing as chlorides and bicarbonates. Burmester, Scott, and Card (1940) studied the uptake of the uterine fluids by the egg by placing prematurely expelled eggs in a synthetic uterine fluid. They found that the rate of uptake of fluids is probably greatest when the egg first enters the uterus and that it gradually decreases until it reaches the minimum after 8 hours. They also concluded that the amount of calcium carbonate added to the shell was not the sole factor governing the rate of uptake of uterine secretions.

CHALAZA FORMATION

The chalazae are paired, spirally twisted strands of egg white which are attached to the two poles of the yolk in line with the long axis of the egg. Hansen (1933) pointed out that the chalazae are made up of materials laid down in the upper magnum but that they are not differentiated until a much later stage of egg formation. Alnquist (1936) discussed the mechanics of chalaza formation. Conrad and Phillips (1938) found the differentiation of the chalazae and the inner thin white to be simultaneous and probably due to the same mechanism. They reported that the inner thin white had a much lower concentration of mucin fibers

than that found in the thick viscous white enclosing the inner thin portion. They therefore proposed that the mechanical segregation of mucin fibers to form the chalazae is responsible for the reduction of mucin in the remaining fluid which becomes the inner thin white. While the egg is in the upper portion of the magnum and the white secreted adjacent to the yolk is still viscous, the mucin content is similar to that of the thick white found in the laid egg. The change in the nature of the albumen immediately surrounding the yolk is not well understood. Almquist (1936) suggested that it is due to a process of syneresis. Tarchanoff's early experiment (1884), frequently repeated since, demonstrated that an amber ball when passed down the oviduct would stimulate the production of normal enclosing structures. This proves that the later differentiation of the white adjacent to the yolk into chalazae and inner thin white is not due to any chemical action of the yolk itself. Almquist (1936) and Conrad and Phillips (1938) state that, since the chalazae in a laid egg are twisted, it seems quite likely that the egg is rotated around its long axis while it is in the uterus. These workers point out that the yolk tends to float with the germ spot on top and, at the time the chalazae are formed, the yolk is surrounded by a semi-fluid suspension of mucin fibers and is therefore free to turn within the white. Under these conditions, it seems probable that, if the egg were rotated, the yolk would remain with the germ spot up while the white moved around it, the mucin fibers being wrapped around the yolk to form the chalaziferous layer and twisted at the ends to form the chalazae. This development in the egg white was duplicated artificially (Conrad and Phillips) by placing interecepted isthunian eggs in a mechanical device. Burmester and Card (1939) found chalazae in yolkless eggs and called attention to the fact that this could not be easily explained by the foregoing theory of chalaza formation. Scott and Huang (1911) approached the problem of chalaza formation histologically and were able to confirm data obtained by other methods. They found evidence that the first steps in differentiation of the chalazae occurred in the posterior division of the magnum. These histological observations seem to confirm the foregoing theories on the mechanics of chalaza formation.

MEMBRANE FORMATION

It has long been recognized that the major function of the isthmus is the secretion of the two egg membranes, the inner one of which is only one-third as thick as the outer (Hays and Sumbardo, 1927). The membranes are comprised of a profuse network of protein fibers which Calverly (1933) found to be keratin. The shell membranes seem to be formed in the isthmus by discrete deposition. The process is initiated as soon as the egg enters the isthmus, and an egg which is half in the magnum and half in the isthmus has a membrane on one end only. Richardson (1935) expressed the view that the inner membrane is formed as the egg moves into the isthmus and that the egg then becomes stationary for a short period while the outer membrane is deposited. The uterus appears to play no part in membrane formation; Pearl and Surface (1909) inserted material into the uterus and found that a calcareous shell and no membrane was formed around it. The air cell forms as a mechanical response to the contraction of the egg contents upon cooling after oviposition. Mihailescu (1934) made observations on the formation of the air cell resulting from an accumulation of air between the two membranes; he found no air cell at the time of oviposition but observed that its formation usually occurred within an hour thereafter. Environmental temperature was found to influence the rate of air-cell formation. Almquist (1933) calculated that in a 2-ounce egg an air space more than $\frac{1}{2}$ inch in diameter would form merely as a result of cooling to 60° F. Further increase in size of the air cell may take place as a result of shrinkage due to evaporation. Asmundson (1939a, 1939b) found the shell membrane of the turkey egg to be relatively much heavier than that of the chicken.

SHELL FORMATION

After the plumping of the membranous egg subsequent to its entrance into the uterus, the next obvious change in the egg is the formation of the shell. Burmester, Scott, and Card (1939) investigated the rate of shell formation by manually expelling

eggs that had been in the uterus from 1 to 20 hours. They found the rate of calcium carbonate deposition to be relatively slow for the first 3 hours that the egg is in the uterus, after which the rate of deposition accelerated. It was suggested that the plumping of the uterine egg acted as a stimulus to shell deposition. Taylor and Martin (1929) have listed a number of environmental, nutritional, and hereditary factors which influence the thickness of the egg shell. Warren and Schnepel (1940) found evidence for the depressing influence of high external temperatures on shell formation. Birds kept at temperatures encountered during the summers in the southern half of the United States produced thinner-shelled eggs. Berg (1945) reported that the shells of the first and last eggs in clutches of three or more were thicker than those in intervening positions in the clutch. He explained the increased thickness of the shell of the last egg of the clutch by the greater time it spends in the oviduct. First eggs of the clutch also had smoother shells than other eggs in the clutch. Asmundson and Baker (1940) reported from the study of several species of birds that the percentage of shell decreases significantly with an increase in the size or volume of the egg.

Warren and Conrad (1942) confirmed the findings of Burmester, Scott, and Card regarding the rate of shell deposition and made additional observations on pigment deposition in brown-shelled eggs. Fischer and Kogl (1923) and Fischer and Lindner (1925) found that the pigment in egg shells is largely porphyrin. By comparing the pigment of prematurely expelled eggs with that of eggs laid by the same hens, Warren and Conrad found that 50 to 74 per cent of the pigment in brown-shelled eggs is added in the last 5 hours before oviposition. This would indicate that pigment deposition is greatly accelerated in the last few hours before laying. Turkey eggs also were found to acquire their speckles very nearly at the time of laying. The vagina, which connects the uterus with the hen's cloaca, was once thought to contribute to shell color, but Asmundson (1931) performed fistulas which eliminated the passage of the egg through the vagina without affecting the characteristics of the egg. It would, therefore, seem that the vagina serves only as an orifice through which the egg is released.

Gutowska, Parkhurst, Parrott, and Verburg (1943) concluded from careful studies that phosphatase activity plays a role in egg-shell formation and that its activity takes place in the blood and not in the shell gland. In shell formation the shell gland (uterus) probably acts only as an organ secreting calcium. Gutowska and Mitchell (1945) proposed that carbonic anhydrase acts as a catalyst in the shell gland for the decomposition of carbonic acid, thus allowing carbonate ions to be formed from bicarbonate ions. These carbonate ions are then utilized in calcium carbonate deposition, the calcium being derived by the shell gland from calcium proteinate which is present in the blood. Hinshaw and McNeil (1943) and Scott, Jungherr, and Matterson (1944) have shown that the feeding of sulfanilamide to chickens exerts an inhibition on the secretory ability of the shell gland. Coles (1938) investigated the cause of so-called hair cracks in chicken egg shells. He found that the incidence of hair cracks varied regionally and suggested that the type of soil and vegetation present might be influencing factors.

Stewart (1935) proposed that pores in the shell were formed as a result of fibers from the shell membranes projecting through the harder portions of the shell. Marshall and Cruickshank (1938) pointed out that the pores were too regularly distributed and placed at too nearly a right angle to the surface to be caused by the projections of interwoven fibers in the membranes.

Almquist (1934) has described the cuticle as being a collagen-like protein. Conrad and Scott (1938) advanced the hypothesis that the cuticle may be formed by soluble proteins which diffuse out of the egg white through the membranes and shell.

OVIPPOSITION

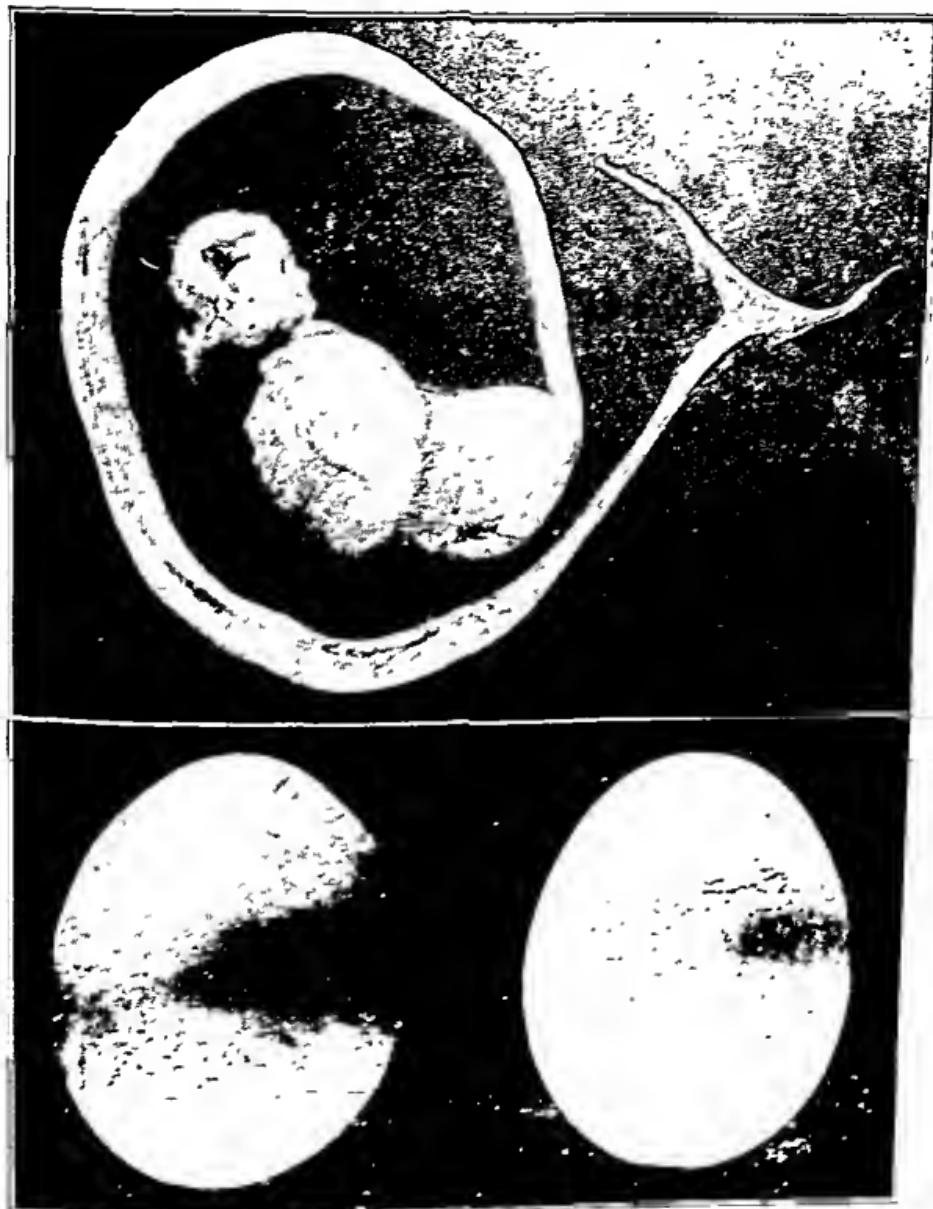
In females with a high rate of laying, the time of oviposition can be predicted fairly accurately from observation of the record of past performance, since such birds maintain a relatively uniform interval between ovipositions. It is known that disturbances, such as moving to unfamiliar quarters, may cause a bird to delay oviposition for several hours. Scott (1940) described such a delayed oviposition, which resulted in the crowding into the uterus of the egg whose oviposition was delayed together

with the succeeding egg, as a consequence of which the latter was misshaped (fig. 18).

Rothchild and Fraps (1944) showed that the ruptured follicle influenced the time of oviposition. They removed the ruptured follicle while the egg that had its origin in this follicle was in the oviduct. In some experiments they removed the next oldest ruptured follicle also. The removal of the ruptured follicle usually resulted in 1 to 7 days' delay in oviposition. It would appear then that in the hen the ruptured follicle plays an important role in determining when the egg carrying its previously contained ovum will be laid. These workers also obtained some evidence that the follicle to ovulate immediately after oviposition has some influence on the time of this oviposition. Bernier (1947) reported that the last egg of clutches of three or more eggs shows more embryonic development than eggs in clutch positions intermediate between the first and last. The degree of embryonic development at laying has been demonstrated to be associated with the length of time spent in the oviduct. Bernier suggested that the delay in oviposition of the last egg in the clutch may be due to the lack of the stimulus accompanying the ovulation of a succeeding egg and that thus evidence which supports the findings of Rothchild and Fraps is provided.

Riddle (1921) first reported that administration of whole posterior pituitary substance would cause premature expulsion of birds' eggs. Later, Burrows and Byerly (1942) found that intravenous injections of obstetrical pituitrin would cause premature expulsion of eggs at any time after they entered the uterus. Oviposition occurred approximately 3 minutes after injection. The nature of the mechanism initiating oviposition is not exactly known, but the foregoing experiments indicate that both the pituitary gland and the ruptured follicle influence the process.

As soon as the shape of the egg can be distinguished, it is evident that the egg remains oriented with the pointed end foremost while it is in the magnum and isthmus. That this position sometimes becomes reversed later has been established by many workers who have observed that either the large or small end of the egg may be laid first. Olsen and Byerly (1932) reported that 85 to 90 per cent of the eggs in the uterus were in a position with



the pointed end posteriorly. They found that only 70 to 80 per cent of these same hens laid their eggs with the small end first and suggested that some eggs become reversed in the act of laying. The reversal of the position of the uterine egg has been observed under the fluoroscope (Warren, unpublished). It should be noted that some eggs show very little difference in the pointedness of the two ends.

ABNORMALITIES IN EGG FORMATION

Numerous abnormalities in egg formation have been recorded. Asmundson (1931) discussed some types of abnormalities. One common aberration is internal laying, in which ovulation occurs, but for some reason the infundibulum of the oviduct fails to pick up the ovum. The cause may be a diseased or abnormal structure of the oviduct. Operations on the anterior end of the oviduct have resulted in this type of failure in egg formation. The accumulation of ova in the body cavity which accompanies this abnormality may result in peritonitis.

The premature expulsion of eggs after injection of pituitary hormones has already been discussed. Thin-shelled eggs found at the roosts in the morning are usually the result of premature expulsion, the cause of which is not known.

Delayed oviposition has already been discussed—it may cause misshaped eggs if the delay is sufficient for the second egg to reach the uterus before oviposition occurs.

The causes of double-yolked eggs have been investigated by Conrad and Warren (1940) using injections of a dye to mark the growth of the ovum. They decided that about 65 per cent of double-yolked eggs result from simultaneous development and ovulation of two ova (fig. 19). Another 25 per cent are caused by the simultaneous ovulation of two ova which have been developed in a normal sequence, one of which should normally have been ovulated a day later. The remaining 10 per cent result either from successive development and simultaneous release of two ova, one of which should have been released a day earlier, or from a day's delay in pick-up of an ovum released in normal sequence. It appears then that double-yolked eggs result from

the simultaneous growth of two ova, from a premature or delayed ovulation of one ovum, or from a delay in the picking up of an ovum.

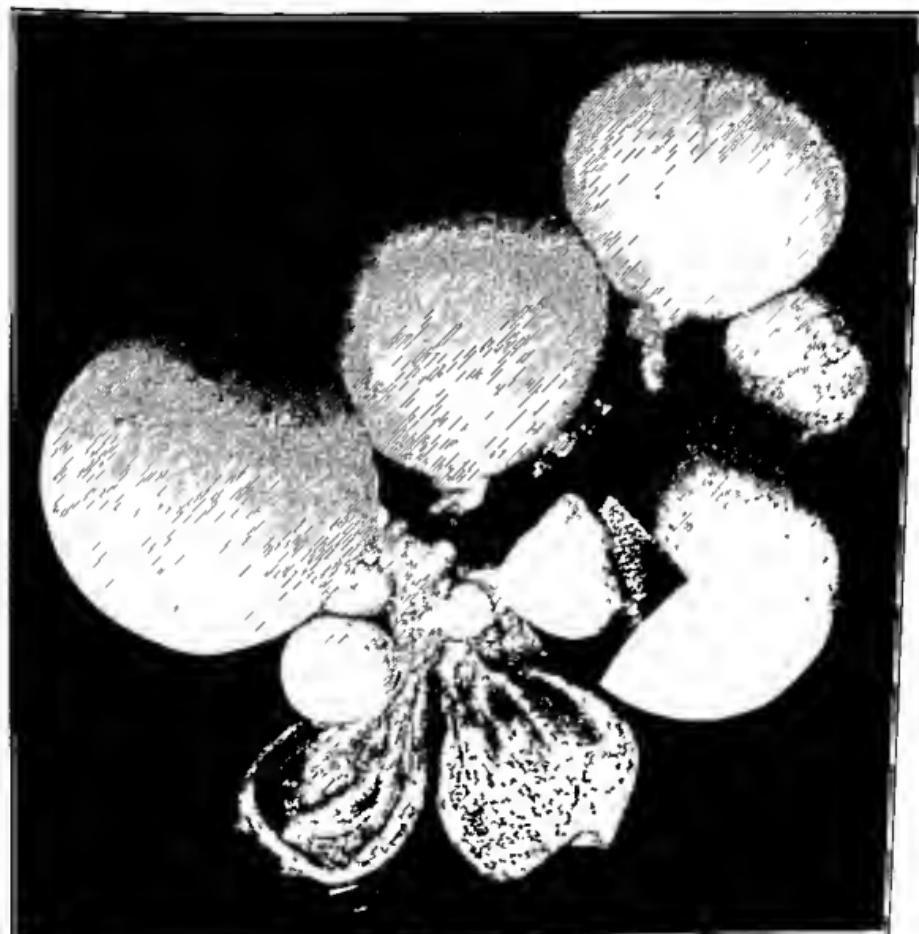


FIG. 19. The ovary of a hen carrying a double-yolked egg in the isthmus. The two ruptured follicles of equal size indicate that this double-yolked egg was the result of simultaneous ovulation. Other causes of double-yolked eggs are listed in the discussion. The smaller, partially resorbed ruptured follicle had carried the ovum enclosed in the egg laid just prior to the double ovulation

envelopes. However, Burmester and Card (1939) resected portions of the anterior end of the oviduct and obtained numerous yolkless eggs, most of which carried no fragments of foreign bodies that could have stimulated white formation.

Misshaped eggs are known to result from abnormalities in the structure of the oviduct, which may be the result of disease or injury. The numerous operative studies of the oviduct, particularly those performed on the posterior portion, have resulted in abnormally shaped eggs. The flattening and wrinkling of the shell of eggs has been accounted for by constrictions in the walls of the isthmus and uterus by Asmundson (1931) and by the presence of two separate eggs in the uterus by Scott (1940).

Blood-spotting in eggs is another abnormality which causes heavy losses in market eggs. Workers at the Illinois Agricultural Experiment Station have given considerable study to this problem. Burmester and Card (1938) concluded that most so-called meat spots are merely blood clots that have degenerated. Nalbandov and Card (1944) found that some blood clots are due to hemorrhages that occur before ovulation. They found that when hens were confined there was a distinct increase in the incidence of blood spots as the season advanced from December to July and that the number of blood spots was found to diminish rapidly when hens were turned out on range. No specific vitamin was found which seemed to influence the production of blood spots. Lerner and Smith (1942) and Jeffrey and Pino (1943) presented evidence that the tendency to produce blood spots is inherited.

Abnormalities involving the enclosure of a completely formed egg within another shell are occasionally reported. Curtis (1916), Roberts and Card (1929), and Romanoff and Hutt (1945) describe such abnormalities. They are usually due to some irregularity in the process of egg formation involving a reversal of peristalsis; thus the egg is caused to traverse portions of the oviduct twice.

FACTORS DETERMINING EGG SIZE AND SHAPE

The size of eggs of wild birds is reported by Amadon (1943) to be a nonlinear function of body size, affected by such factors

as clutch number, condition of the young at hatching, natural selection, and the artificial selection of domestic birds.

Differences of considerable magnitude in the size of eggs occur among individuals and among strains of poultry. The Manchurian hen, for example, is reported to lay single-yolked eggs weighing as much as 4 ounces (Kohmura, 1931). Although size is an individual trait, there are minor variations among the eggs of any hen. Numerous workers have reported that the size of a pullet's egg increases for several months after she starts to lay. Some effort has been made to relate the mechanism of egg formation to variations in egg size. Heredity is a factor in determining egg weight, but the explanation of how this hereditary trait is expressed is to be found in the physiology of egg formation. The weight of an egg is obviously merely the sum of the weights of its parts, and so one must examine the factors influencing the various egg components to find the solution of the general problem of egg size. Numerous workers have published data on the size relationships of the egg parts (Scott, 1938; Asmundson and Baker, 1940; Asmundson, Baker, and Emlen, 1943). It has long been known that the size of the yolk is an important factor in determining egg weight. Asmundson and Jervis (1933) presented evidence that the size of the oviduct influenced the amount of secretion. Asmundson (1939a, 1939 b) found a higher percentage of shell membrane and a lower percentage of shell in the turkey egg than in the hen egg. He attributed this variation in proportion to differences in the comparative length of the isthmus and of the uterus in these two species. Scott (1938) worked with several species of domestic birds and found a positive relationship between body size and yolk weight. He also included six breeds of chickens in his studies and found that the Black Minorca, with less than average body size, had larger eggs and larger oviducts than any other breed. The yolk weight of the Black Minorca egg was less than that of the other breeds studied, but the white weight was much greater. Heritable variations of this magnitude in the ratio of egg parts have a definite economic bearing on the practice of merchandising frozen yolks or frozen whites. A wide differential has existed between the value of processed yolks and whites.

explanations for variations in the shape of the egg must be sought. The fact that the pointed end (if one exists) of the egg is foremost in passage through the oviduct suggests that the mechanics of propulsion may be responsible. The available data do not offer any satisfactory solution of this problem and emphasize the need for further work on the problem.

RELATION OF EGG FORMATION TO HATCHABILITY

The mechanism of egg formation necessarily determines the physical characteristics of the resulting egg. The hen's egg consists largely of protective and nutritive parts which make it possible for the fertilized ovum to develop into a viable individual. Irregularities in the process of egg formation may have deleterious effects on the hatchability of the egg. Birds in general differ from higher animals in that the developing embryo is carried within the body of the mother for only a very short time and thus is deprived of the nutrition and protection which in mammals is provided by the mother during gestation. An excellent summary of the influence of the physical characteristics of the egg on hatchability has been published by Landauer (1941).

Although the following discussion will clearly demonstrate that the characteristics of the egg do affect the well-being of the developing chick, a number of fallacious ideas have developed regarding the relation of the visible characteristics of the egg to the resulting chick. For instance, it was believed for a long time that the shape of the egg indicated the sex of the chick it carried—a belief which long since has been refuted. Attempts have also been made to predict fertility by shell characteristics, but there is no critical experimental evidence to support the possibility. The determination of both sex and fertilization occur in the early stages of egg formation, and the possibility of using any external features of the egg for such predictions is thus eliminated.

Rate and Period of Laying

The rate at which eggs are being formed, as well as the length of the period during which a female has been laying, has been the

Various workers have recorded the fact that clutch position influences the size of the eggs of individual hens, the first egg of the clutch being the largest. Scott (1938) found that these differences in egg size were due almost entirely to the amount of egg white present.

Bennion and Warren (1933) reported that high environmental temperatures tend to reduce egg size. Their study showed that all components of the egg decreased in size with high temperatures but that the shell and white decreased considerably more than the yolk. This was interpreted to indicate that the oviduct was more sensitive to the effects of high temperatures than was the ovary.

The writer, in operative work, has often observed that the egg shape was quite clearly defined at the time the forming egg entered the isthmus. The pointed end of the egg was always observed to be in the direction of egg movement, an orientation which could be seen most clearly in sharply pointed eggs.

Numerous observations on egg shape have involved resections of the oviduct. However, such an approach to the solution of the problem of egg shape is not too dependable, since any malformation of the tube through which the egg passes after being enclosed in a membrane is likely to leave its impression on the egg whether or not this region normally has any effect on egg shape. Harper and Marble (1945a) concluded that the quality of the albumen had no influence on the shape of the egg. They (1945b) also failed to find any differentiation in the musculature of the oviduct that would account for observed differences in egg shape. Measurements of the parts of the oviducts of hens differing from each other in the shape of the eggs laid revealed no evidence of a relationship between egg shape and the size of the oviduct. Harper and Marble (1945b) have suggested that the supporting oviducal ligaments may influence the shape of the egg. Data were collected (Warren, unpublished) to determine whether the rate of movement (as determined by interval length) of the egg through the oviduct was related to egg shape, and no such evidence was found. The observation that eggs acquire shape even before the membrane is deposited suggests that shape is determined in the magnum. Since Harper and Marble failed to find any association of the musculature or size of this region with egg shape, other

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Rate and Period of Laying

The rate at which eggs are being formed, as well as the length of the period during which a female has been laying, has been the

subject of numerous investigations. Poultrymen have believed that excessive egg production is detrimental to the hatching power of the egg. Lamson and Card (1920) found no influence of the pullet-year production upon the second year's hatchability. Their results were confirmed by studies of Knox (1927), Jull (1928), Bronkhorst (1933), and Funk (1934b). Warren (1934) obtained some evidence that the number of eggs laid by Rhode Island Red pullets before March showed a slight positive correlation with hatchability but found no similar association in White Leghorns or in Rhode Island Red hens.

Several workers have found that a high intensity of laying during the hatching season seems to be favorable to hatchability. Data supporting this view were published by Jull (1930); Byerly, Titus, and Ellis (1933); and Funk (1934b). McNally and Byerly (1936) found, however, that eggs laid at intervals of 27 hours had maximum hatchability. Bernier (1947) found an interval range from 23 to 27 hours to be optimum for hatchability.

Size of Egg

Because of its practical significance, numerous studies have been made on the problem of the influence of egg size on hatchability. The results of these studies prove conclusively that in a given flock of chickens the largest eggs have the poorest hatchability. There are some differences in results, probably because all workers did not use the same standard for large egg size. Halbersleben and Mussehl (1922) reported that large eggs hatched less well than did either small or medium-sized eggs. Dunn (1922) found that the hatchability of large eggs of both hens and pullets was below the average of all eggs. Jull and Haynes (1925), Axelsson (1932), Warren (1934), Funk (1934b), Godfrey (1936), and Shibata and Murata (1936) all presented data supporting the view that the largest eggs hatch poorly. Jull and Haynes (1925) and Warren (1934) each found that small eggs hatched better than medium-sized eggs, but this observation was not confirmed by other workers. Byerly and Marsden (1938) found that the weight range of 73 to 78 grains gave best hatchability in turkey eggs, with larger or smaller

eggs hatching less well. Insko, McLaury, and Baute (1943) found that the average turkey egg size of 71 to 98 grams hatched better than either smaller or larger eggs.

Byerly (1934) and McNally and Byerly (1936) made the interesting observation that the larger eggs produced by a group of birds required a slightly longer incubation period.

Shape of Egg

Benjamin (1920) found no influence of egg shape on hatchability, and Jull and Haynes (1925) carried out similar studies with results that supported this finding. Again, Hays and Sumbardo (1927) found no relationship between either egg length or egg width and hatchability. Thus, from the few investigations reported, it would seem that variations in egg shape as ordinarily encountered do not affect the hatching power of the egg.

Yolk Characteristics

Various characteristics of the egg yolk have been studied in relation to the hatchability of the egg. Bronkhorst and Hall (1935) found no influence of the relative amounts of yolk solids upon hatching power. Hall and Van Wagenen (1936) studied the yolk-shape index in relation to hatchability without finding any significant correlation. Rudy and Marble (1939) also found no evidence that hatchability of eggs was influenced by variations in yolk weight. Scott and Warren (1941), although also finding no influence of yolk size on hatching power, did find that the ratio of yolk weight to white weight had an effect on hatchability; eggs in which the average proportion of white to yolk was 2 to 1 had a higher hatchability than eggs having a higher or a lower proportion of these two components.

Barancheev (1936) studied the influence of variations in yolk color in the eggs of a flock kept under uniform conditions. He found that the three arbitrary grades of color had a descending hatching power, with the lightest-colored yolks having the poorest hatchability. The birds laying eggs with light yellow yolks may have been those that failed to assimilate some of the nutrients of the feed with a resulting decrease in the viability of the embryos.

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that loss of weight per unit area of shell exerted any influence on hatchability.

Several workers have used the specific gravity of the egg as a measure of shell thickness, since it provides a rapid means of measuring this quality. Mussell and Halberslben (1923) found a significant positive correlation between specific gravity and hatchability. Munro (1940) found a somewhat curvilinear relationship between this character of the egg and its hatching power. He had definite evidence that eggs with low specific gravity hatched poorly and recommended this test for predicting hatchability. Phillips and Williams (1944) found no relationship between specific gravity and the hatchability of turkey eggs.

The results of studies on the influence of the porosity of the shell on hatchability are somewhat conflicting; however, Landauer (1941) has suggested that varying incubator conditions may explain these conflicts, since some modern incubators regulate the humidity so accurately that the deleterious effect on hatchability from excessive porosity is overcome.

Clutch Position

A clutch includes all eggs laid on successive days. Hens laying at short intervals consequently have relatively long clutches. Hutt and Pilkey (1930) found evidence that eggs laid during the afternoon had poorer hatchability than those laid in the morning. They explained these results on the assumption that morning eggs would be at a considerably more advanced stage of development when laid than afternoon eggs. This assumption was based on the fact that the morning group included a higher percentage of eggs which followed a break between clutches and that such eggs had been held in the oviduct overnight instead of being laid in late afternoon. Pritsker (1940) confirmed the findings of Hutt and Pilkey regarding the comparative hatchability of eggs laid in the morning and in the afternoon. These workers found this difference in hatchability to be more pronounced in March and April. Hays (1936, 1937) found no evidence that the hour of laying had any influence on hatching power. Funk (1934a), working with considerable data, drew conclusions that were the opposite of those of the workers previously cited; he concluded that eggs laid in the afternoon

White Characteristics

The so-called standing-up quality of egg white has been the subject of numerous investigations largely because it is one of the best measures of commercial egg quality. In some of the studies of this measure of egg quality, investigation has been made of the effects of these variations on hatchability. Van Wagenen and Hall (1936) and Hall and Van Wagenen (1936) carried out a series of studies in which they found evidence that the higher-scoring albumen improved hatching power. Godfrey (1936) obtained low negative correlations between the weight of thick albumen and hatchability. Since the relative amount of thick albumen largely determines albumen quality, these results are in contradiction to those of Van Wagenen and Hall. Godfrey also found a negative correlation between total albumen weight and hatchability, but this probably reflects the detrimental effect of large egg size on hatching power. Wilhelm (1939) and Rudy and Marble (1939) failed to find any influence of albumen quality on hatchability. The foregoing results, then, as a whole are conflicting and inconclusive.

Shell and Shell-Membrane Characteristics

The shell characteristics necessarily influence the viability of the enclosed embryo since the shell protects the embryo, supplies the necessary calcium for its development, and provides a means of gas exchange between the developing chick and its environment. Dunn (1922-1924) found that visibly porous shells had a high water loss during incubation and had low hatchability. Hays and Sumbardo (1927) found no evidence that hatchability was influenced by either shell thickness or the number of pores in the shell. Axelson (1932), using as a measure of porosity the weight loss in the 24-hour period before the eggs were placed in the incubator, found that the eggs that had the least loss in this period had the thickest shells and the highest hatchability. Shibata and Murata (1936) also found that eggs which had the greater weight loss gave the poorer hatchability. Wilhelm (1939) found a low positive correlation between shell thickness and hatchability. Mueller and Scott (1940) obtained no evidence

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hatched better than eggs laid in the morning. The experiments of McNally and Byerly (1936) had results similar to Funk's findings, and so we have these several, apparently critical, investigations with exactly opposite results.

The length of time between the ovipositions of the last egg of one clutch and the first egg of the succeeding clutch is approximately 16 hours greater than that between successive eggs of any one clutch. Scott and Warren (1936) showed that the assumption that the first egg of the clutch spends 16 hours more time in the oviduct than subsequent eggs of a clutch is incorrect. By palpation of the oviducal egg through the vent these workers could not detect that the first egg of the clutch spent any more time in the oviduct than other eggs and suggested that the delay in the laying of the first egg is due to a delay in ovulation and not to the retention of this egg in the oviduct.

Development of Egg at Time of Lay

Since fertilization probably takes place immediately after ovulation, the chick embryo is several hours old at the time of oviposition. Warren and Scott (1936), McNally and Byerly (1936), and Bernier (1947) are agreed that the extent of development at the time of oviposition is dependent upon the time spent in the oviduct (interval length). Taylor and Gunns (1935, 1939), and Bernier (1947) calculated the relative degree of embryonic development at oviposition by measuring the diameter of the blastoderm of the unincubated egg, whereas Warren and Scott (1936) and McNally and Byerly (1936) based their calculation on somite count after a period of incubation. Taylor and Gunns (1935), McNally and Byerly (1936), and Bernier (1947) all found evidence that the first egg of the clutch has slightly greater embryonic development than later eggs of the clutch. Scott and Warren (1936) were unable to confirm the results of earlier workers regarding this problem. Atwood (1929), Berg (1945), and Bernier (1947) are in agreement that the last egg of a clutch spends a longer time in the oviduct. Berg found that the last egg of the clutch had a thicker shell, and Bernier that it had greater embryonic development than eggs preceding it.

McNally and Byerly (1936) and Bernier (1947) have each found evidence for a curvilinear relation between the extent of

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CHAPTER 3

Fertility in Chickens and Turkeys

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INTRODUCTION

The reproductive efficiency of domestic fowls is determined by the number of eggs laid, the percentage of these eggs that are fertile, and the percentage of fertile eggs that hatch into chicks. This chapter deals with fertility, the second of these factors.

The problem of obtaining a high percentage of fertile eggs is of economic importance to poultry raisers and hatcherymen. It is estimated that 10 to 15 per cent of all eggs incubated are in-

fertile. Because incubated infertile eggs have a reduced value as food and require additional incubator space and labor they represent a considerable economic loss. Information concerning the nature of fertilization and the various factors affecting it should, therefore, be of particular interest to the hatchery industry. The published research on the subject is considerable, but much of it is fragmentary, and in many instances it has been published in journals not generally available to hatcherymen and breeders.

FERTILIZATION

Fertilization is defined as the union of the sperm from the male with the egg or ovum of the female. Although several sperms may penetrate the cell wall of the egg, only the nucleus of one sperm unites with the nucleus of the egg in the fertilization process. The others are called supernumerary sperms and disappear between 4 and 5 hours after fertilization (Patterson, 1911). Patterson also observed that hens' eggs were fertilized immediately after ovulation when the egg was in the funnel part of the oviduct. Recent observations reported by Olsen (1942) revealed that the hen's egg is normally fertilized in the funnel within 15 minutes after it is released from the ovary.

The manner in which sperms get from the vagina to the funnel—the entire length of the oviduct—has been the object of several interesting studies. Warren and Kilpatrick (1929) found spermatozoa in all parts of the oviduct 6 hours after mating. They also observed that many of the sperms had lost their whip-like tails after 6 hours, and that after 24 hours after mating, sperms with tails intact were rarely found. Walton and Whetham (1933), on the other hand, were unable to find sperms in the oviducts of females after mating, although the females laid fertile eggs for as long as 15 days thereafter. Fertility persisted after irrigation of the peritoneal cavity and of the oviduct with spermicidal solutions. Van Drimmelen (1945b) found normal, native spermatozoa in the infundibulum or funnel of the fowl's oviduct up to 14 days after insemination. In other studies (Van Drimmelen, 1946) he observed that the sperms were located in "sperm-nests" in crypts or gland ducts in the mucous lining of the funnel region of the oviduct, sometimes as many as 50 to 80 sperms being

found in one duct. This observation affords a plausible explanation for the persistence of fertility following irrigation of the oviduct with spermicidal solutions.

Parker (1931) observed that in the oviducts of birds there is a narrow band of cilia which beats toward the front end of the oviduct in contrast to the general ciliary motion which is directed to the rear. He suggested that sperms deposited in mating were transported through the vagina and uterus of the oviduct by anti-peristaltic muscular action, after which they were transported through the remainder of the oviduct by the pro-ovarian ciliary tract described above. Swimming action of the sperms also may aid in the migration through the uterus since sperms have been found to be motile in uterine fluid at body temperatures of fowls (Munro, 1938a). Moreover, Munro observed that sperms were immotile in juices from the magnum and funnel of the oviduct at temperatures corresponding to the body temperatures of the fowl.

Investigations by Mimura (1939) revealed that sperms inseminated in the rear end of the oviduct may reach the funnel and the ovary in as short a time as 26 minutes. When an egg was present in the oviduct sperms were retained in the inseminated area until the egg was laid. When inseminations were made after laying, rapid progress of the sperms toward the funnel was observed.

In general practice, fertility is determined by incubating eggs for a period of several days or longer and then candling them. Eggs containing live or dead embryos as evidenced by a germ spot, blood vessels, or blood ring are classed as fertile, and all clear eggs as infertile. Munro and Kosin (1945) presented evidence that in many of these clear eggs embryonic development was stopped before the egg was laid, a condition they refer to as preovipositional embryonic death. This may be related to the somewhat common observation of hatcherymen that when fertility is low hatchability is also low. Since the blastodisc of the fertile egg differs from that of the infertile egg in both size and structure (fig. 20), several investigators, including Barfurth (1895) and Kosin (1944b; 1945), have shown that fertility can be detected in newly laid, broken-out eggs without the aid of a microscope.



FIG. 20. Fertile and infertile unincubated blastodises of egg yolks. The fertile germ spot on yolk (A) is enlarged and shows definite rings of organization. The infertile germ spot on yolk (B) is smaller in size, has a cloud-like center surrounded by clear vacuoles, and lacks rings of organization. Courtesy C. A. Gunns, University of California.

A practical method of determining fertility in unincubated eggs without breaking them out would be of great value to the poultry industry.

FERTILITY IN THE MALE DOMESTIC FOWL

Anatomy of the Male Reproductive System

The anatomy of the reproductive system of the female domestic fowl has been described in the preceding chapter. A knowledge of that material will be a great aid to a clear understanding of parts of this chapter.

The reproductive system of the male chicken consists of two testes with epididymides, the vasa deferentia (or sperm ducts), and the copulatory apparatus (fig. 21). Descriptions have been given by several investigators including Bradley (1915) and Burrows and Quinn (1937). The two testes are bean-shaped and lie on either side of the vertebral column in the abdominal cavity. They are situated below the front lobes of the kidneys, and in the mature male the testes are partially surrounded by the membranes of the posterior thoracic and the abdominal air sacs. Cowles and Nordstrom (1946) have presented evidence that the air sacs serve to cool the testes of blackbirds during spermatogenesis. That the scrotum serves this purpose in mammals is well known.

The testes are pale yellow (Sisson and Grossman, 1938), but occasionally a pigmented or partially pigmented testicle is observed (Bittner, 1925). Numerous blood vessels are seen on the testes. Testes from the general-purpose breeds of chickens are larger than those from Leghorns. Parker, McKenzie, and Kempster (1942a) found that the average weight of the Leghorn testicle was 9.5 grams, whereas in heavy breeds the average was 15.5 grams. Mitchell, Card, and Hamilton (1926) found that the weight of both testicles in White Rock cockerels weighing 7 pounds was 33 grams. It is apparent from these figures that the combined weight of the testes comprises about one per cent of the total weight of the adult male domestic fowl.

Microscopic study of histological preparations shows that the bulk of the testes is composed of thousands of seminiferous tubules

(fig. 22). It is in these tubules that the germ cells are changed into sperms by the process of spermatogenesis. The sperms are found in clusters with the tails projecting into the centers of the tubules and the heads attached to Sertoli or nurse cells (Gray, 1937). When the sperms are mature, the heads become detached

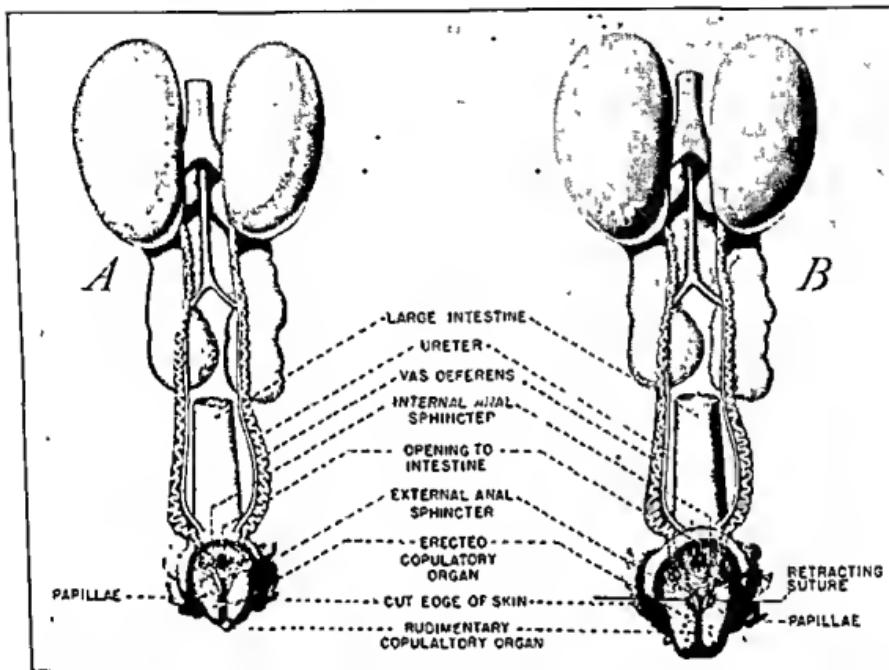


FIG. 21. Reproductive organs of the male chicken *A* and the turkey tom *B*. Note that the rudimentary copulatory organ of the cock has one apex whereas that of the tom has two. The courses of the sperm ducts (*vasa deferentia*) in the wall of the cloaca are indicated by dotted lines. Courtesy U. S. Dept. Agr. and Poultry Science.

and the sperms pass through the tubules into the excurrent ducts of the epididymis.

The interstitial or intertubular cells of the cock testis are homologous with the Leydig cells or interstitial cells of the mammalian testis according to Nonidez (1924). In adult male fowls these cells are few and confined chiefly to the larger intertubular spaces. There is considerable evidence that the interstitial cells secrete the male sex hormone in mammals, but in fowls the site of secretion of this hormone is controversial. The work of Brene-

man (1938) indicates that in the male fowl the hormone is secreted by the seminiferous tubules.

The epididymis is small and rudimentary in comparison to that of other farm animals. After the sperms pass through the

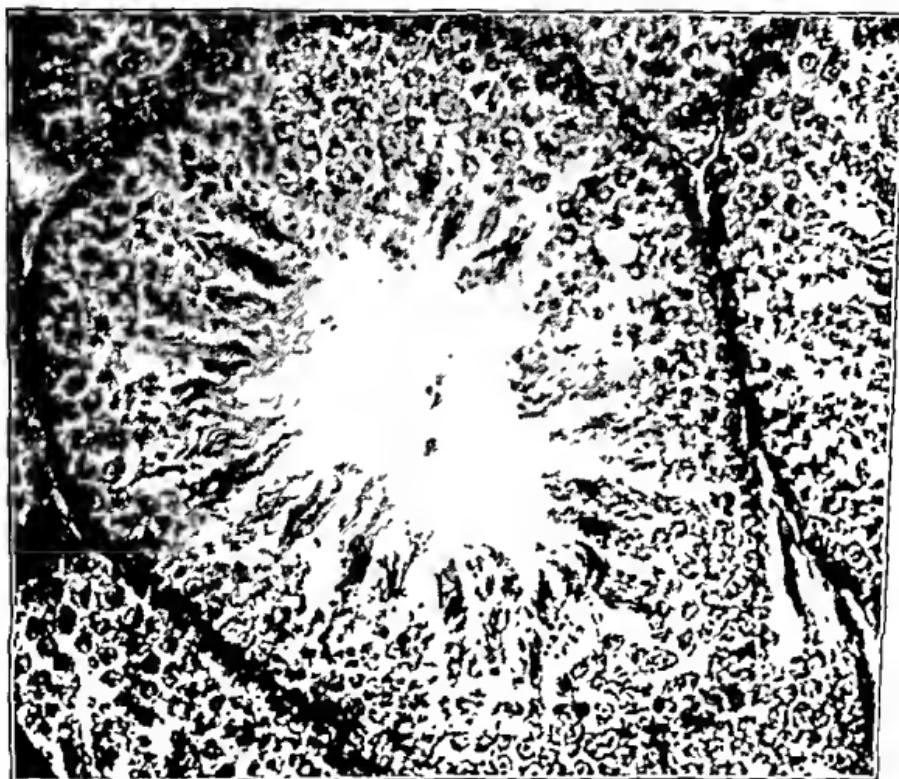


FIG. 22. Section of testis tissue showing cross section of seminiferous tubule. Note the activity of the reproductive cells and the clumps of spermatozoa attached to the Sertoli or nurse cells. *Courtesy Missouri Agr. Expt. Sta.*

ducts of the epididymis they enter the vas deferens. Each vas deferens extends from the epididymis to the cloaca and is situated near the middle of the back close to the vertebral column. As the ducts continue backwards they become larger in diameter until at the point where they enter the wall of the cloaca the diameter measures as much as 3.5 millimeters in some individuals (Parker *et al.*, 1942a). Burrows and Quinn (1939) have expressed the opinion that semen is temporarily stored in

this enlarged or bulbous portion of the vas. Figure 23 shows a cross section of the epididymis.



FIG. 23. Section through the epididymis. *D*, efferent ducts of epididymis; *S*, seminiferous tubules of testis. Courtesy Missouri Agr. Expt. Sta.

Those interested in the histology of the testis, epididymis, and the vas deferens are referred to the excellent description given by Gray (*op. cit.*).

The copulatory apparatus of the male domestic fowl consists of two papillae and the rudimentary copulatory organ (fig. 24). Gross anatomy has been described by Burrows and Quinn (1937), and gross and microscopic anatomy by Parker *et al.* (1942a).

The sperm ducts enter the walls of the cloaca and terminate in two small, conical-shaped papillae. Each papilla has a hole through which semen is emitted during copulation. The rudimentary copulatory organ is situated immediately to the rear of the papillae. There is no hole or lumen in this organ.

Avian spermatozoa have been described by a number of investigators including Bradley (1915), Warren and Kilpatrick (1929) and Parker, *et al.* (1942a). Avian sperms differ from mammalian sperms in having slender, cylindricial heads which are equipped anteriorly with distinct, sharp-pointed structures called aero-somes (fig. 25). Adamstone and Card (1934a) observed globules of fat and fatty acid material in the sperm head and suggested that these globules might serve as stored food material.

Development of Fertility

During the late summer and fall months relatively large percentages of infertile eggs are often produced from matings in which yearling or older cocks are used. With the increase in the amount of fall hatching found in some sections of the United States, this infertility results in considerable economic loss. The possibility of using cockerels from early spring hatches as breeders makes it desirable to have information on the development of fertility in young cockerels.

Latimer (1924) observed that there was similarity between the growth curve of the testes of growing White Leghorn cockerels and the four-phase curve found in the growth of mammalian testes. There was (1) a period of slow growth in the first 50



FIG. 24. Copulatory apparatus of the male domestic fowl showing rudimentary copulatory organ *R* and papillae *P* ($\times 3\frac{1}{2}$). Courtesy Missouri Agr. Expt. Sta.

days, succeeded by (2) a period of slightly increased growth from 50 to 80 days of age, then (3) a period of rapid or pubertal growth from 210 to 260 days of age, and lastly (4) a period of little change after sexual maturity. Mitchell, Card, and Hamilton (1926) found that in White Rock cockerels the average weight of both testes increased from 0.1 gram at 4 weeks to 33

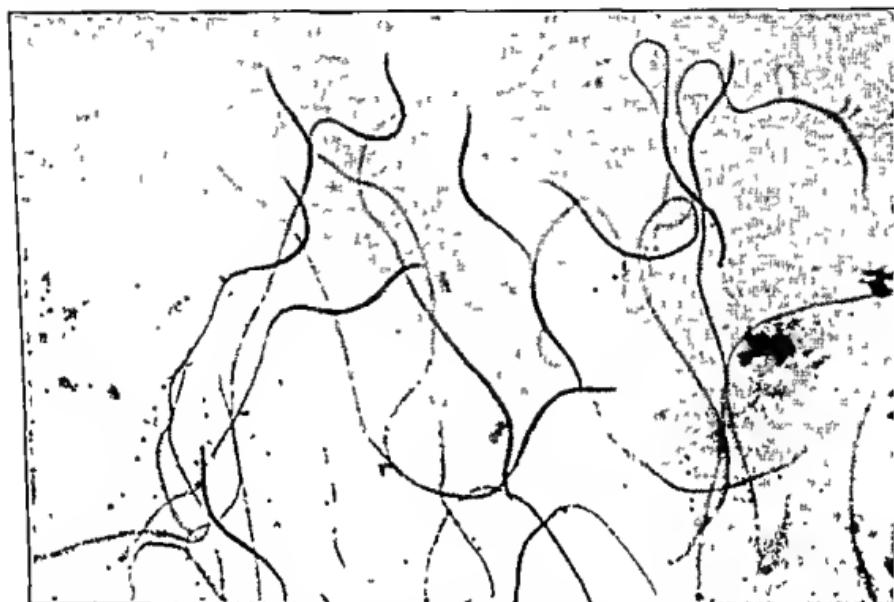


FIG. 25. Spermatozoa of the domestic fowl. Courtesy Kansas State College and Poultry Science.

grams at 46 weeks. There was relatively little growth of the testes until the cockerels approached weights of 6 to 7 pounds. In Los Baos Caatoaese fowls, Froada and Marcelo (1938) observed a gradual increase in testicular and ovarian weights to 6 months of age, and from 6 to 8 months the weight increases were rapid. The growth of testes from White Leghorns and New Hampshire cockerels from 1 day to maturity was studied by Parker, McKeazie, and Keiperster (1942b). The period of most rapid testicular growth in both breeds was from 8 to 12 weeks. Until 12 weeks of age the testes of Leghorns and New Hampshire cockerels increased in weight at about the same rate, but from 16 to 24 weeks the testes of the Leghorns were much heavier (fig. 26).

Bennett (1947) observed that the weights and dimensions of

the male gonads increased steadily to 125 days of age and that the ovary of the female increased to 131 days. Beyond these ages there tended to be a decline in gonad weights, although

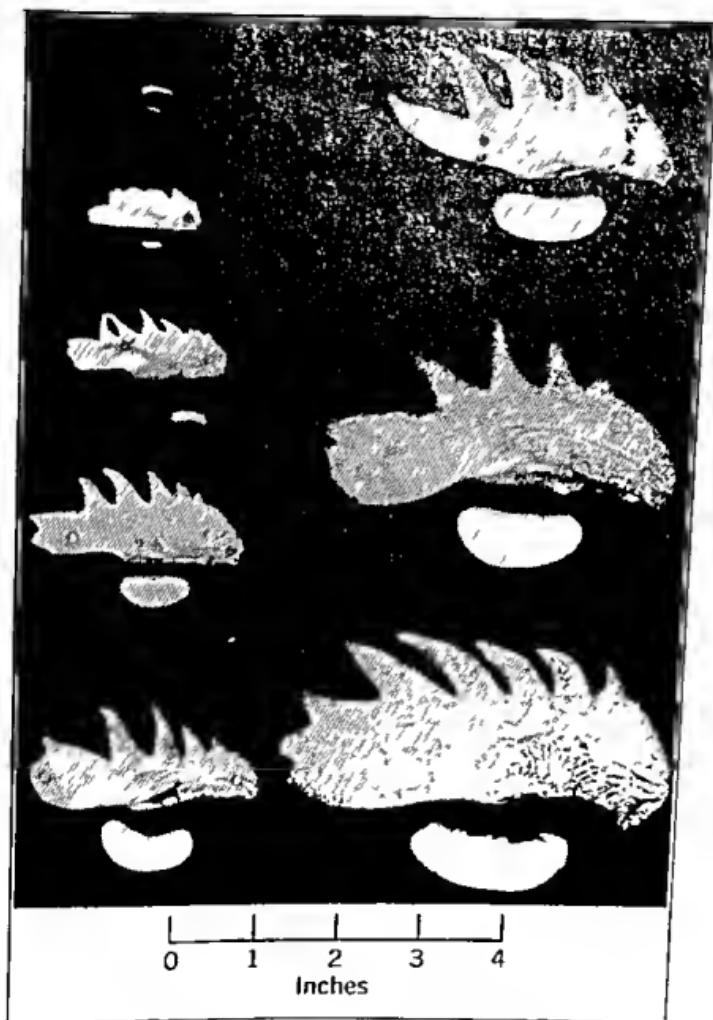


FIG. 26. Comb and testis growth of White Leghorn cockerels by 4-week periods from time of hatch to 21 weeks. The last group is from an adult male. Courtesy Missouri Agr. Expt. Sta. and Poultry Science.

males from early hatches did not show this decline in testis size. Observations reported by Byerly and Knox (1916) and Greenwood and Blyth (1916) show that the sexual development of pullets hatched late in the season also is not so rapid as that of pullets from early hatches.

Several studies have been made of changes in the histology of the testes with age. Boring (1912) observed that the seminiferous tubules in testes of day-old cockerels were much smaller than those in the testes of older cockerels. No signs of dividing germ cells were observed in the chick testes. Observations on Barred Rock cockerels, based upon histological examinations of testicular tissues and individual matings, indicated that some males reached sexual maturity at 16 weeks. However, in pen matings a satisfactory level of fertility may not be attained until the cockerels are 26 weeks old (Hogue and Schnetzler, 1937). Although spermatozoa were observed in testicular tissues of White Leghorn and New Hampshire cockerels at 12 weeks by Parker *et al.* (1942b), the males were not capable of fertilizing hens to an appreciable extent in mating trials until they were 24 weeks old. They also observed that diameters of the seminiferous tubules increased from $35\ \mu$ at 1 day to $250\ \mu$ at maturity. As judged by histological changes and the volume of semen produced at 12 weeks, White Leghorn cockerels of a strain high in fecundity reached sexual maturity at earlier ages than cockerels from a strain low in fecundity (Jones and Lamoreux, 1942). Spermatozoa were observed in a testis from one male of a highly fecund strain at 8 weeks. Sampson and Warren (1939) obtained semen capable of fertilization from a White Leghorn cockerel between 9 and 10 weeks old.

It is generally known that the comb of the male fowl is a secondary sex organ and that its size is influenced by the male sex hormone secreted by the testes. In developing cockerels it has been observed that the periods of greatest comb growth coincide with the periods of greatest testicular growth (Blyth, 1928; Parker *et al.*, 1942b), an observation which suggests that comb development is indicative of sexual development. Hogue and Schnetzler (*op. cit.*) found that, after young Barred Rock males reached 16 to 20 weeks of age, those with large combs and wattles were more likely to fertilize eggs than those with relatively small combs and wattles. The size of the combs of White Leghorn males at 10 weeks has been shown by Jones (1944) to be a relatively sensitive indicator of spermatogenic activity, and considerable reliance may be placed upon it as a measure of sexual maturity.

Physiology of Semen Production

The functional changes of spermatozoa during their passage through the epididymis and vas deferens of the cock have received little attention. Several investigators have shown that in mammals spermatozoa, during their passage through the epididymis, undergo a maturing or ripening process in which they acquire the ability to move and to fertilize ova. Munro (1938b) gave a comprehensive review of this phase of the physiology of mammalian sperm. In his work with the male fowl, Munro observed that when spermatozoa from the testis were used in artificial insemination they were practically incapable of fertilization; when fluid from the epididymis was used, 12.8 per cent of the inseminated females produced some fertile eggs, and, when semen from the lower vas deferens was used, 74 per cent of the hens were fertilized. There was a corresponding increase in the motility of sperms from the three regions. In the sexually active male, sperms pass through the excurrent ducts in as short a time as 24 hours. It is interesting to note that approximately the same length of time is required for the egg to pass through the oviduct (chap. 2).

In other studies, Munro (1938c) found that sperms remained alive in the vas deferens for an average of 26 to 28 days, irrespective of the presence or absence of the testes.

Semen from domestic fowl varies in appearance from a dense, opaque white suspension which has a high sperm concentration to a clear, watery fluid with low sperm concentration (Purker, McKenzie, and Kempster, 1942a). A summary of characteristics of semen from the domestic cock is shown in table 3. There is a great deal of variation in data reported by the various investigators, depending upon the techniques employed and perhaps also to some degree upon the animals used and the length of time between ejaculates. Semen volumes were less when the fluid was intercepted during mating than when it was collected by the abdominal massage technique described by Burrows and Quinn (1937). Intercepted ejaculates averaged less than 0.4 cubic centimeter whereas most of those collected by the massage technique were between 0.5 cubic centimeter and 1.0 cubic centimeters. Less variation was observed in the concentration of sperms in

the semen. The average density reported by all workers was about 3.5 millions of sperms per cubic millimeter of semen. As measured by the number of sperms intercepted during mating, the average number of sperms per ejaculate was slightly less than a billion, whereas the average number of sperms obtained per collection by the massage technique was 2.3 billion.

TABLE 3

SUMMARY OF CHARACTERISTICS OF SEMEN FROM DOMESTIC FOWLS OBSERVED BY SEVERAL INVESTIGATORS

Investigator	Method of collection	Amount per collection (cc.)	Sperm density (sperms per cu. mm. of semen) (millions)	Total sperms per collection (billions)	Kind of fowls
<i>For chickens:</i>					
Payne (1914)	From cloaca of female	2-5.5 Av. 2.9	White Leghorn
Craft, McElroy, and Penquite (1926)	From cloaca of female	2-4	White Leghorn
Hutt (1929)	Intercepted with watch glass	0.11 (gm.)	0.02-8.8 Av. 4.0	Brown Leghorn
Burrows and Quinn (1937)	Ejaculation by massage	1.0	Crossbred
Munro (1938)	Ejaculation by massage	0.5	1.9-10.2	Brown Leghorn
Parker, McKenzie, and Kempster (1942a)	{ Intercepted in semen collector Ejaculation by massage	0.05-1.0 Av. 0.36	0-10 Av. 3.1	0-5.3 Av. 0.9	New Hampshire
Wheeler and Andrews (1943)	Ejaculation by massage	0.3-1.5 Av. 0.88	0.03-11.2 Av. 3.4	0.01-15 Av. 3.3	New Hampshire
<i>For turkeys:</i>					
Burrows and Marsden (1938)	Ejaculation by massage	0.3-0.4	Bronze
Parker (1946a)	Ejaculation by massage	0.2-0.6 Av. 0.33	3.6-12.7 Av. 8.4	1.3-5.4 Av. 2.8	Broad Breasted Bronze

Observations on hydrogen-ion concentration of cock semen have been reported by Parker *et al.* (1942a), and by Wheeler and Andrews (1943). The range was from pH 5.3 to pH 8.5. The former investigators found the average pH to be 7.27 whereas the latter group observed it to be 7.04.

Motility of sperms, or their capacity to show movement outside the chicken, has been observed by a number of workers, including Payne (1914), Grodzinski and Marchlewski (1935), Burrows and Quinn (1939b), Shaffner, Henderson, and Card (1941), and Wheeler and Andrews (*op. cit.*). Sperms may be kept alive for

a period of several hours to as long as 15 days. In general, sperms remained motile longer at storage temperatures of 1° to 10° C. The excellent motility maintained by sperms when stored is apparently not associated with their fertilizing capacity, since Burrows and Quinn found that when semen was stored at 4.4° C. or lower the sperms lost their fertilizing ability in 2 hours or less. Shaffner (1942) reported that motility persisted in semen preserved at -79° C. for 14 months. Hens inseminated with semen held at the above temperature for 1 hour laid 12 fertile eggs out of 48, but none hatched.

In experiments on the relation of semen or sperm characteristics to fertilizing capacity Hutt (1929) found no correlation between sperm concentrations and the males' capacities to fertilize hens' eggs when the number of sperms per cubic millimeter of semen was between 825,000 and 7,000,000. According to Sampson and Warren (1939), few male fowl produce semen containing large numbers of abnormal sperms; however, one male that did was sterile. They likewise observed that the association between the sperm concentration of semen and fertility was not very pronounced. The work of Munro (1938d) showed that 100,000,000 sperms must be inseminated for optimum fertility, and Parker *et al.* (1942a), indicated that as long as this number of sperms was inseminated the sperm concentration of the inseminating fluid had little influence on fertility. The latter group also reported evidence that fertility was positively correlated with the motility of fresh semen and negatively correlated with the number of abnormal sperms in the semen. Shaffner and Andrews (1947) found that there were high degrees of positive correlation between fertility and the initial motility of sperms, between fertility and the survival period of sperms at 5° C., and a negative correlation between fertility and the reduction time of methylene blue, which is a measure of the consumption of oxygen by semen.

Further investigations directed toward predetermining the fertilizing capacity of breeding males should be encouraged since additional information on the subject would aid in the solution of a problem of considerable economic importance to the poultry industry.

The sexual activity or libido in the male domestic fowl has been observed by several investigators. In accordance with what

one would expect, a great deal of variation was observed among individual males and, therefore, among the results reported by different workers. Heuser (1916) found that the number of copulations per male per day in White Leghorns ranged from 0 to 32, and over a 14-day period from 16 to 235. Also in observations of White Leghorn males, Penquite, Craft, and Thompson (1930) reported that the number of matings per day ranged from 6 to 28. The maximum number of copulations per day with Leghorn males was found by Philips (1918) to be 41 and by Martin and Anderson (1918) to be 34—however, only 10 of the 34 females mated were fertilized.

Certain factors were observed to influence sexual activity. One such factor was preferential mating (Heuser, *op. cit.*; Upp, 1928). Receptivity of the females and the number of females in the mating were other conditions which influenced sexual activity (Philips, *op. cit.*; Martin and Anderson, *op. cit.*; Guhl, Collias, and Allee, 1945). In most experiments the males were found to be more active sexually during the late afternoon than at other times of the day (Heuser, *op. cit.*; Upp, *op. cit.*; Parker, McKenzie, and Kempster, 1940).

That sexual activity is not a reliable index of the male's reproductive capacity has been indicated by the studies of Craft, McElroy, and Penquite (1926), who observed that sexually active birds often produced a higher percentage of dead or weak sperms than less active males. When n number of successive ejaculates were intercepted during attempted matings, n tendency toward a decrease in volume, density, and total number of sperms with each succeeding ejaculate was observed (Parker *et al.*, 1940). Very few sperms were observed in the fluid from some males after 3 to 4 ejaculations. These results suggest that sex drive in fowls beyond certain limits does not increase fertility and may be detrimental to it. General appearance and body type were not found to be reliable indexes of sexual activity (Raimo, 1942).

Factors Influencing Semen Production

Although the relation of age to semen production is of practical importance, very little information has been reported on the subject. As previously mentioned, Sampson and Warren (1939) obtained semen that was capable of fertilization from a White

Leghorn cockerel between 9 and 10 weeks old. Jones and Lamoreux (1942) collected semen from 36 of 42 twelve-weeks-old White Leghorn cockerels. In a high-fecundity strain the average volume was 0.13 cubic centimeter and in a low-fecundity strain, 0.045 cubic centimeter. Collections taken at 24 weeks approached in volume the semen collected from mature males. Collections were not reported for intermediate ages. Lorenz and Lerner (1946), also working with White Leghorns, collected semen from 28 per cent of their cockerels at 13 weeks, from 35 per cent at 14 weeks, from 57 per cent at 15 weeks, and from 60 per cent at 16 weeks. Barred Rock cockerels attained a relatively high level of fertility at 7 months; however, full production of spermatozoa was not approached until 8 to 9 months (Parker and McSpadden, 1942).

It has been observed that White Leghorn cockerels in the fall of their first year produced semen that was less dense and contained a smaller percentage of live spermatozoa than semen produced the following spring (Penquite, Craft, and Thompson, 1930). During the second breeding season, however, the males were less productive than during the first.

Observations by a number of investigators, including Whetham (1933), show that egg production in the hen is influenced by season. Higher egg production has been found to be correlated with increasing length of day. Because of the similarity in the hormonal mechanisms governing egg production in the female and semen production in the male, it was not unexpected when Parker and McSpadden (1943a) and Wheeler and Andrews (1943) found that season had a definite influence on the production of spermatozoa. Both groups found that the total number of sperms ejaculated by cockerels increased from December through April. The former workers also observed that, like egg production, semen production declined during the late spring and summer months (fig. 27). The decline in fertility late in the season is probably related to this seasonal slump in sperm production. Since an average temperature of 83.5° F. had no deleterious effect on sperm production (Wheeler and Andrews, *op. cit.*), it appears that length of day is the determining factor.

Although spermatozoa are produced in the tubules of the testes throughout the day, there is evidence that spermatogenic activity

is greater at certain times of the day than at others. Riley (1940) observed that the tubules were most active at 3 A.M., whereas Macartney (1942) found spermatogenic activity to be greatest at midnight.

A tendency for the density of the sperm suspension to decrease during the day with successive matings has been reported by

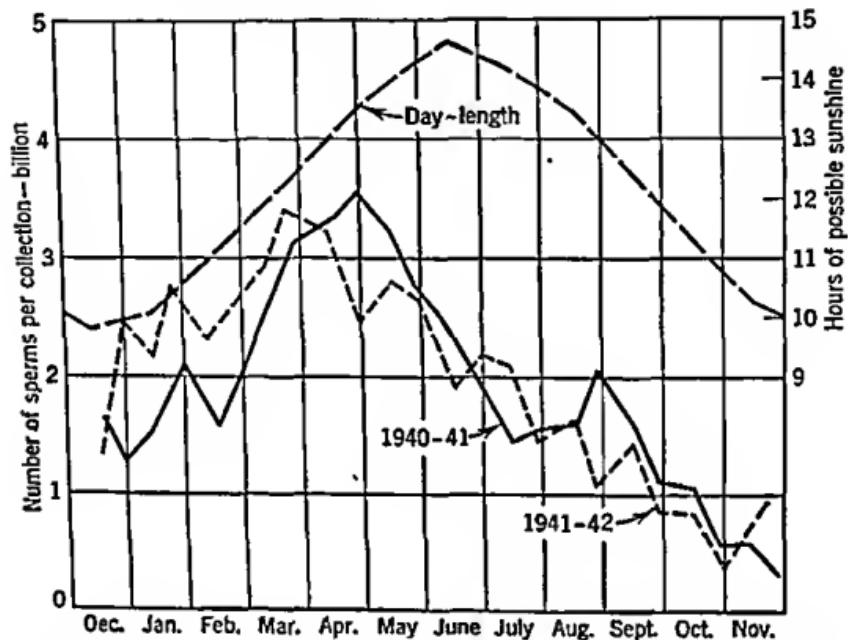


Fig. 27. Relation to season of sperm production of Rhode Island Red cockerels. Like egg production, sperm production appears to be influenced by length of day. Courtesy Tennessee Agr. Expt. Sta.

Penquite, Craft, and Thompson (*op. cit.*). When semen was collected at hourly intervals during the day, semen quantity and sperm density were found to decrease (Sampson and Warren, 1939). When semen was collected at intervals ranging from 6 hours to 8 days, it was observed (Parker *et al.*, 1942a) that sperm density, total numbers of sperms per collection, and, to a lesser extent, semen volume increased with the lengthening of the intervals between collections.

Although some studies have been made on the relation between nutrition and sperm production, considerably more research should be conducted on this important problem. Craft, McElroy,

and Penquite (*op. cit.*) reported that males that were on deficient rations produced fewer spermatozoa than those on more adequate rations. Maslieff and Zabiakina (1935) noticed that volume and density of semen increased when males were fed silage. Adamstone and Card (1934b) observed that some males became sterile after being fed diets deficient in vitamin E for prolonged periods. Definite structural changes in the heads of the spermatozoa of male fowls on this diet occurred in a short time. Research to date indicates that the vitamin A content of the feed has little influence on the volume of semen produced by male fowls (Burrows and Titus, 1938). Restriction of feed consumption sufficient to cause losses in body weights was shown to affect sperm production and the fertilizing capacity of cockerels adversely (Parker and McSpadden, 1943b).

Benoit (1935), working with ducks, and Margolf (1940), with turkeys, demonstrated that increments in the daily amount of light induce marked testicular enlargement and spermatogenesis during seasons when the reproductive organs of these two species are more or less quiescent. Results of experiments reported by Lamoreux (1943) show that amount of light also influences sperm production in the male chicken. Males exposed daily to 12 hours or more of light produced significantly greater volumes of semen than males that had been exposed for less than 1 hour daily (fig. 28). Evidence was presented to show that the threshold of response lies between 9 and 12 hours of light daily. Maximum response to stimulation by light was reached in about a month, and the duration of the response varied with individual males from less than 4 months to longer than 9 months.

Removal of the pituitary gland was observed by Hill and Parkes (1934) to cause a rapid atrophy of the testes and cessation of spermatogenesis. Injection of extract from the pituitary increased the production of spermatozoa in normal White Leghorn cockerels (Nikolaevsk and Mnw, 1942). The gonadotropic principle of pregnant mare's serum may have a stimulating effect on sperm production in male fowls. Shlossner and Smyth (1944) and Hnys (1945) reported that pregnant mare's serum may be used effectively on sexually inactive male fowl. The influence of the male sex hormone on the fertility of males was studied by

Koch (1936). Males injected with hormone fertilized a significantly greater percentage of eggs than did noninjected controls.

Results reported by Crew (1925), working with chickens, and by Jaap (1933), working with ducks, indicate that the thyroid favorably influences spermatogenesis. Recently Blivaiss (1947) demonstrated that the removal of the thyroid retards the growth

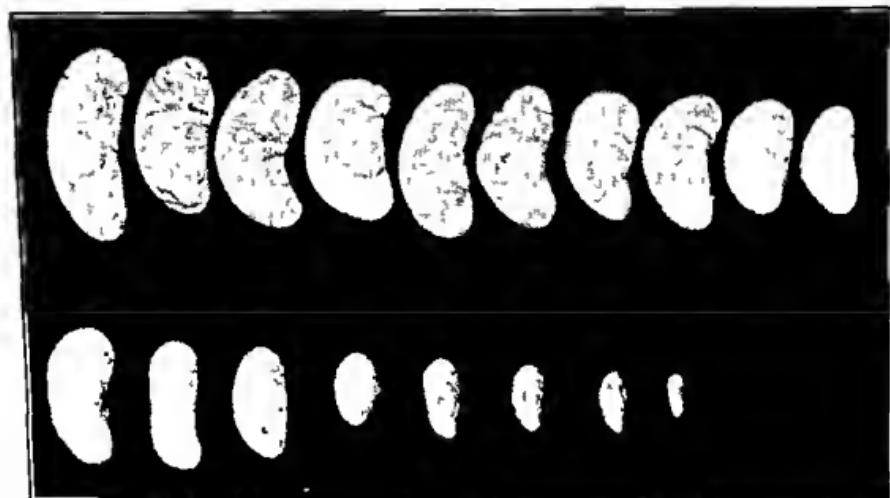


FIG. 28. Influence of amount of artificial lighting on testis size. Photograph of the largest testis from each mature White Leghorn male exposed to 14 hours of light daily (top row), January 18 to June 8; and to less than 1 hour of light daily (bottom row), December 3 to June 8. Courtesy *Journal Experimental Zoology and Cornell Univ.*

of the gonads and the secondary sexual characters in male fowls. Thiouracil, a thyroid depressant, has been shown by Shaffner and Andrews (*op. cit.*) to reduce the quality and fertilizing capacity of semen. The injection of adrenalin was found to reduce semen production and to cause degeneration of the germinal tissue (Wheeler, Scarey, and Andrews, 1942).

Further experimentation on the relation of hormones to reproduction, particularly in old males, should yield results of great benefit to poultry breeders.

Dubbing, or the removal of most of the comb and wattles from males, is a common practice (fig. 29) (Lamoreux and Joaas, 1912). Dubbed males are less apt to be injured by freezing and also appear to be more active than undubbed males of the

large-combed breeds. Payne and Ingram (1927) observed that a high percentage of infertile eggs occurred when the males' combs were frozen. Several workers, including Buckner, Insko, and Martin (1933) and Marlow and Payne (1940), observed an increase in weight of the testis following dubbing. More recent

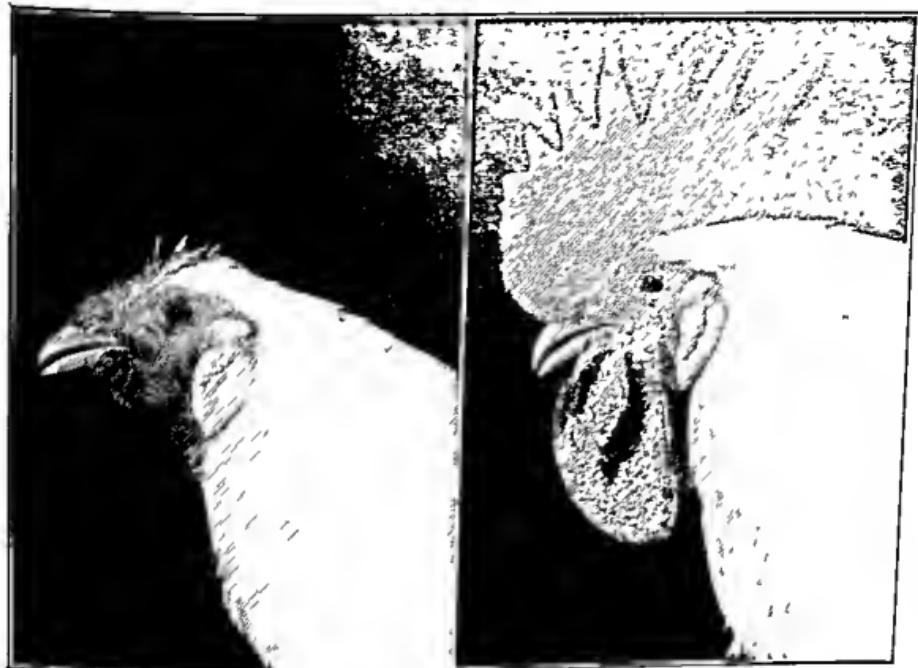


FIG. 29. Many hatchery men and breeders find that dubbing improves fertility. Dubbing prevents frost injury. Dubbed males have no large combs to interfere with feed consumption. Male on right undubbed, male on left dubbed. *Courtesy Cornell Univ. and Poultry Science.*

studies reported by Landauer (1942), Lamoreux and Jones (1942), Scarcey and Andrews (1943), and Jones (1944) demonstrate, however, that the removal of combs and wattles does not increase size of the testis or semen production. It would appear that dubbing has little or no effect on the fertility of male fowls unless the combs interfere with normal feed consumption and activity or unless they are injured by freezing.

In studies reported by Jones and Lamoreux (*op. cit.*), it was observed that cockerels from a high-laying strain of White Leghorns produced significantly more semen at 12, 24, and 30 weeks than males from a low-fecundity strain. Investigations by

Lorenz and Lerner (*op. cit.*) show that age of sexual maturity, as measured by semen production, is inherited in White Leghorn cockerels.

Hutt (*op. cit.*) studied a group of males from which various amounts of the testicular tissue had been removed by operation and found no relationship between the number of sperm ejaculated and the weight of gonadal tissue remaining. His analysis of the data of Craft, McElroy, and Penquite (1926) showed no correlation between size of the testis and the concentration of sperm in the semen. On the other hand, Burrows and Titus (1939), Lamoreux (1943), and Jones (1944) found significant positive correlations between testis weights and semen production. Data published by Burrows and Titus (*op. cit.*), Parker, McKenzie, and Kempster (1940), and Jones (*op. cit.*) show that such characteristics as body weight, general appearance, and willingness to mate were not reliable indexes of semen production.

In a group of 78 cockerels, Dove (1928) observed 4 which had no copulatory organs, and 3 of the 4 were sterile. Maedonald and Taylor (1933) observed no copulatory organs in hens or capons and found that slips had smaller organs than normal males. They also showed that male and female chick embryos differed with respect to the size of the copulatory organ after 12 days of incubation. Thus it appears that the development and relative size of the organ are influenced by testicular activity.

The influence of X rays upon reproduction in male fowls has been investigated only to a limited extent. Rolf, Schroeder, and Higgins (1934) demonstrated that exposure of 6-week-old cockerels to X rays of 1750 to 2700 r.* reduced the development of the testes and of the combs and wattles. X-ray treatment of fowl sperms (Kosin, 1944a) reduced their fertilizing capacity, and the exposures of sperms to 5544 to 6488 r. completely destroyed fertility.

FERTILITY IN THE FEMALE DOMESTIC FOWL

Onset and Duration of Fertility

The onset of fertility, or the time elapsing between mating and the laying of the first fertile egg, is of interest to hatcherymen

* r. = symbol for roentgen, international unit of radiation.

and poultry breeders. Under natural mating conditions the phenomenon has been observed by a number of investigators. Reviews on the subject have been published by Nieolaides (1934) and Jull (1940). Several workers, including Philips (1918), Fronda (1926), Dunn (1927) and Nieolaides, show that the first fertile egg may be laid in as short a time as 19½ to 23 hours after mating. This is interesting when considered in relation to the fertilization process and the time required for egg formation (chap. 2). In a flock of White Leghorns, Waite (1911) found that by the fourth day after the introduction of the males 70 per cent of the eggs were fertile, and Crew (1926) reported results that indicate that the maximum fertility in flock matings may be expected by the end of the first week.

The length of time in which hens continue to lay fertile eggs after natural mating has been observed by a number of workers, and the literature on the subject has been reviewed by Nieolaides (*op. cit.*) and by Jull (*op. cit.*). The duration of fertility after the removal of the male ranged from as short a time as 5 days to as long as 32 days. The average hen, however, remained fertile for only 11 to 15 days, with some variation in the several studies. It would appear that a fair level of fertility may be expected for a week to 10 days after removal of males from the flock; however, some drop in percentage of fertile eggs may be observed after 3 to 5 days.

Munro (1938d) found that hens artificially inseminated in the afternoon laid only 1 fertile egg out of 81 the day after insemination; on the second day, 88 per cent of the eggs were fertile. Parker, McKenzie, and Kempster (1942a) found that after artificial insemination of hens in the afternoon, no fertile eggs were laid the next day; the highest percentage of fertile eggs was reached on the third day, and thereafter fertility declined (fig. 30). The last fertile egg was laid on the twenty-fifth day. Moore and Byerly (1942) reported that maximum fertility was attained on the third and fourth days after artificial insemination and that fertility fell off sharply after the sixth day.

Selective and Competitive Fertilization

Several investigators including Crew (1926), Warren and Kilpatrick (1929), and Bonnier and Trulsson (1939a) have

shown that as a rule the latest insemination is the fertilizing one. When one male replaces another male in mating, the offspring of the new male supplants those of the former within a few days. Warren and Kilpatrick found very little overlapping in the production of offspring of different males.

Dunn (1927) cited data indicating that sperms from males closely related to the females fertilized a higher percentage of

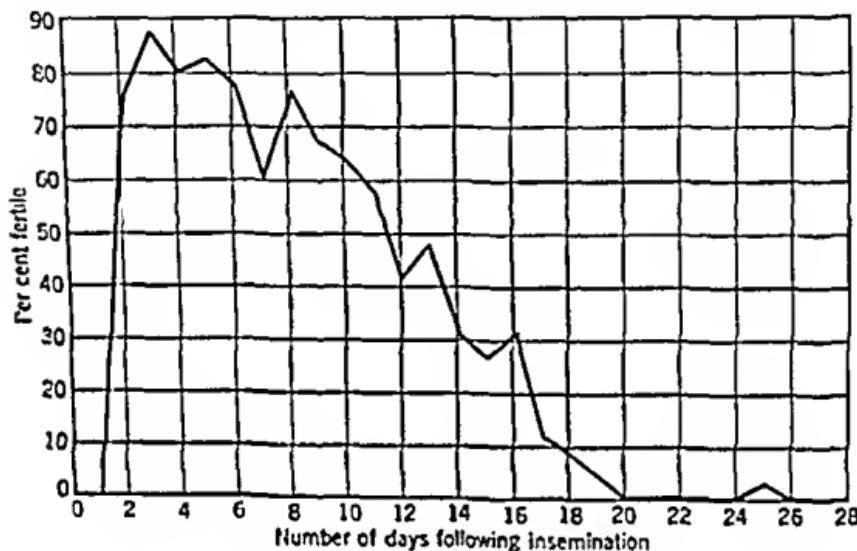


FIG. 20. Duration of fertility after artificial insemination of hens. Courtesy Missouri Agr. Expt. Sta.

Factors Influencing Fertility in the Female

Age is a factor that is related to fertility in female as well as in male domestic fowls. Data of Curtis and Lambert (*op. cit.*) showed that the duration of fertility from a single mating was longer for hens than for pullets. Martin and Insko (1934) found a tendency for fertility to decline with age in hens 2 to 8 years of age. Jull (1935) observed that the fertility of pullets in White Leghorn, Rhode Island Red, and cross-bred birds was higher than in the same birds as yearlings; but in Barred Rocks the reverse was true, although none of the differences were statistically significant. The results of studies over a 15-year period with Rhode Island Reds reported by Hays and Sanborn (1939) showed that females had their maximum fertility as yearlings and exhibited a less marked decrease in fertility with succeeding years than did the males. Insko, Steele and Wightman (1947) found that fertility in White Leghorn and Rhode Island Red hens declined progressively after the first year. Since experimental results in this field are conflicting, further investigation is required before definite conclusions can be drawn.

Several reports of research indicate that the time of day when mating occurs influences the fertility of the eggs subsequently laid. Moore and Byerly (1942) showed that a lower percentage of fertile eggs was laid after the artificial insemination of hens having a hard-shelled egg in the uterus than after the insemination of hens that had just laid or of hens that had no egg in the oviduct. Malmstrom (1943) observed that fertility was only 32.6 per cent when inseminations were made while a hard-shelled egg was in the uterus, and 70 per cent when a membranous egg was in the oviduct. These results suggest that for highest fertility hens should be inseminated during the latter part of the day, since very few hens would have hard-shelled eggs in their oviducts at that time.

Under natural mating conditions Graeewski and Scott (1943) found that fertility was 70 per cent in unrestricted matings, 55 per cent when matings were restricted to forenoon, and 81 per cent when matings were restricted to the afternoon (table 4). In artificial insemination experiments, Parker (1945) found that the percentage of fertile eggs was 49 per cent when hens were

TABLE 5

THE PROPORTION OF INFERTILE EGGS PRODUCED BY THREE GROUPS OF HENS
 THAT LAID 13-22, 23-31, AND 32-40 EGGS, RESPECTIVELY, DURING
 A 6-WEEK PERIOD, 1935-1939
 (Lamoreux, 1940a)

Year	Egg production								
	13 to 22 eggs in 6 weeks			23 to 31 eggs in 6 weeks			32 to 40 eggs in 6 weeks		
	No. of hens	Eggs laid	Infertile eggs (%)	No. of hens	Eggs laid	Infertile eggs (%)	No. of hens	Eggs laid	Infertile eggs (%)
1935	16	320	26.2	102	2,860	18.2	55	1,844	9.9
1936	11	208	41.8	73	2,069	26.8	68	2,301	18.4
1937	34	657	17.8	125	3,460	12.4	41	1,367	11.2
1938	10	200	12.0	93	2,674	8.0	48	1,597	8.8
1939	15	288	24.3	104	2,922	12.6	61	2,046	16.4
Total	86	1,673	24.4*	497	13,985	15.6*	273	9,155	13.0*

* Unweighted average of the percentages for the 5 years.

It has been pointed out by Lamoreux (1940a) that fertility is lower during periods when the rate of lay is low because hens copulate less frequently, as found by Heuser (1916), and have a shorter duration of fertility following insemination. In experimental attempts to increase the sexual receptivity of hens by estrogenic hormones, Lamoreux found that 12 daily injections of 100 to 1000 rat units of Progynon-B had little if any influence on the rate of copulation.

Although the size of the clutch is related to fertility, the position of the egg within the clutch is not (Funk, 1939; Lamoreux, 1940a).

Hays and Snibborn (1939) studied the relation between winter egg production, as measured by the number of eggs laid before March 1, and fertility. In one group of 702 pullets they observed little relationship between egg production before the hatching season and fertility, but in another group there was some evidence that high winter egg production was deleterious to fertility. They stated that their data on the subject were inconclusive and that

TABLE 4

THE INFLUENCE OF RESTRICTING THE TIME OF MATING OF CHICKENS
ON FERTILITY

(From data of Gracewski and Scott, 1943)

Mating	Eggs set	Per cent fertile
Restricted to forenoon	325	54.8
Restricted to afternoon	393	80.9
Unrestricted	201	70.1

inseminated in the morning, 71 per cent following noon inseminations, and 77 per cent following late afternoon inseminations. These reports agree in showing that insemination, either natural or artificial, occurring during the afternoon resulted in higher fertility than when inseminations were unrestricted or restricted to the forenoon. The presence of a hard-shelled egg in the oviduct at the time of mating definitely reduced fertility.

Rate of lay of female fowls influences their fertility. Results reported by Warren and Kilpatrick (1929) and Nicolaides (1934) showed that there was a tendency for the more intense layers to produce a larger percentage of fertile eggs. It was observed by Funk (1939) that hens laying the largest clutches of eggs had highest fertility. Hens laying four-egg clutches produced eggs that were 95 per cent fertile, whereas hens laying one-egg clutches were only 84 per cent fertile. Comprehensive studies were made on the subject by Lamoreux (1940a). He, also, found that the rate of egg laying was positively correlated with the percentage of fertility. Hens laying 13 to 22 eggs in 6 weeks produced more infertile eggs than hens that laid at a more rapid rate (table 5). The proportion of infertile eggs was significantly higher among females that laid clutches of 1 to 3 eggs than among those that laid clutches of more than 3 eggs. Females laying at a rapid rate remained fertile longer following artificial insemination than those laying at a slower rate. Malmstrom (1943) found that under natural mating conditions fertility was higher in more intense layers, but that after artificial insemination fertility was slightly higher in less intense layers. Bernier (1947), also, has shown that females laying at a higher rate than the average have higher fertility.

TABLE 5

THE PROPORTION OF INFERTILE EGGS PRODUCED BY THREE GROUPS OF HENS THAT LAID 13-22, 23-31, AND 32-40 EGGS, RESPECTIVELY, DURING A 6-WEEK PERIOD, 1935-1939
 (Lamoreux, 1940a)

Year	Egg production								
	13 to 22 eggs in 6 weeks			23 to 31 eggs in 6 weeks			32 to 40 eggs in 6 weeks		
	No. of hens	Eggs laid	Infertile eggs (%)	No. of hens	Eggs laid	Infertile eggs (%)	No. of hens	Eggs laid	Infertile eggs (%)
1935	16	320	26.2	102	2,860	18.2	55	1,844	9.9
1936	11	208	41.8	73	2,060	26.8	68	2,301	18.4
1937	34	637	17.8	125	3,460	12.4	41	1,367	11.2
1938	10	200	12.0	93	2,674	8.0	48	1,597	8.8
1939	15	283	24.3	104	2,922	12.6	61	2,046	16.4
Total	86	1,673	24.4*	497	13,983	15.0*	273	9,153	13.0*

* Unweighted average of the percentages for the 5 years.

It has been pointed out by Lamoreux (1940a) that fertility is lower during periods when the rate of lay is low because hens copulate less frequently, as found by Heuser (1916), and have a shorter duration of fertility following insemination. In experimental attempts to increase the sexual receptivity of hens by estrogenic hormones, Lamoreux found that 12 daily injections of 100 to 1000 rat units of Progynon-B had little if any influence on the rate of copulation.

Although the size of the clutch is related to fertility, the position of the egg within the clutch is not (Funk, 1939; Lamoreux, 1940a).

Hays and Sanborn (1939) studied the relation between winter egg production, as measured by the number of eggs laid before March 1, and fertility. In one group of 702 pullets they observed little relationship between egg production before the hatching season and fertility, but in another group there was some evidence that high winter egg production was deleterious to fertility. They stated that their data on the subject were inconclusive and that

additional information was desirable. Observations by Hays (1943) showed that fertility of yearling hens during March and April was not affected by the length of the first laying year. It is a rather common opinion that extremely persistent females are often infertile during the next breeding season.

Spermatoxins or a high titer of spermatozoal antibodies induced in the blood stream of hens by repeated intravenous injections of fowl semen were apparently unimportant as causes of infertility in domestic fowls (Lamoreux, 1940b).

Fertilization in the hen is influenced by the number of sperms introduced in a single insemination. The number of fertile eggs produced has been demonstrated to be affected when the number of sperms was about 100 million or less (Munro, 1938d), and none was fertile when the number fell below 1 million. In later work Munro (1946) suggests that low fertility is not necessarily the fault of the male or of the sperm but may sometimes result from unfertilizable eggs.

FACTORS INFLUENCING FERTILITY INVOLVING BOTH MALE AND FEMALE DOMESTIC FOWLS

Since the ratio of males to females in the breeding flock undoubtedly influences the percentage of fertile eggs produced, one would expect to find considerable experimental data on this point. However, there is not a great deal of material on this subject. Byerly and Godfrey (1937) found that fertility decreased in linear fashion as the number of females per male increased—from about 95 per cent when the ratio was 4 to 1 to about 35 per cent when the ratio was 166 to 1. These studies indicate that the maximum total of eggs fertilized by a single male would be obtained by mating that male to about 120 laying females. Hays and Sanborn (1939) found that a range of 1 to 14 in the number of females mated to each male had no influence upon the percentage of fertile eggs produced. Since this is a problem of considerable importance to the hatchery industry, critical experiments designed to measure the relation of the sex ratio in breeder flocks to fertility of eggs as influenced by age, rate of production, breed, and season of the year should be encouraged.

Preferential mating, or the tendency for the male to mate more often with certain females in the flock than with others, has been observed by Philips (1919), Upp (1928), Warren and Kilpatrick (1929), Hays and Sanborn (1939), and Lamoreux (1940a). It has been shown that males definitely have favorites and that this is often a cause of impaired fertility in single-male breeding pens. Hays and Sanborn showed that males were largely responsible for the low fertility, since a change of males resulted in 93 per cent of the infertile matings becoming fertile. Lamoreux found that artificial insemination of these infertile females resulted in an increase in the percentage of fertile eggs laid from 2.5 to 60.9. Also, Sampson and Warren (1936) observed that 14 of 21 females that were persistently sterile in pen matings became highly fertile when artificially inseminated.

There appears to be very little information relative to the influence of type of mating on fertility. Nicolaides (1934) observed no significant difference in fertility of eggs from stud matings from those from pen matings. However, Bird (1937) and Jeffrey (1944) found that stud mating was unsatisfactory for females confined in batteries, and both workers were of the opinion that artificial insemination was more effective under such circumstances.

Seasonal influence on fertility is well known to hatcherymen and breeders and has been demonstrated experimentally on several occasions (fig. 31). Upp and Thompson (1927) reported data showing that fertility decreased considerably during the summer and fall months. Funk (1938) observed that the fertility of White Leghorns was highest during the spring months—the eggs being approximately 90 per cent fertile at that time—and that it may decrease during the summer as much as 20 per cent below that of spring. Studies on seasonal variation of fertility of Barred Rocks by Parker and McSpadden (1942) and of Rhode Island Reds by Malmstrom (1943) showed that fertility decreased rapidly from April to July. In both experiments the lowest fertility was observed in eggs laid during July.

The work of Heywang (1944) indicates that high temperatures are, at least in part, responsible for the summer decline in fertility. As maximum environmental temperatures increased from 82.8° to 101.8° F. the percentage of fertile eggs laid declined from

94 to 68. Observations at lower temperature ranges have been reported by Hays and Sanborn (1939) and Lamoreux (1942). The former workers found that when the average outside temperature was below 32° the average fertility of Rhode Island Reds ranged between 54 and 77 per cent, and, when the temperature rose to above freezing, fertility ranged from 70 to 85 per cent. With weekly hatches over an 11-week period, fertility rose con-

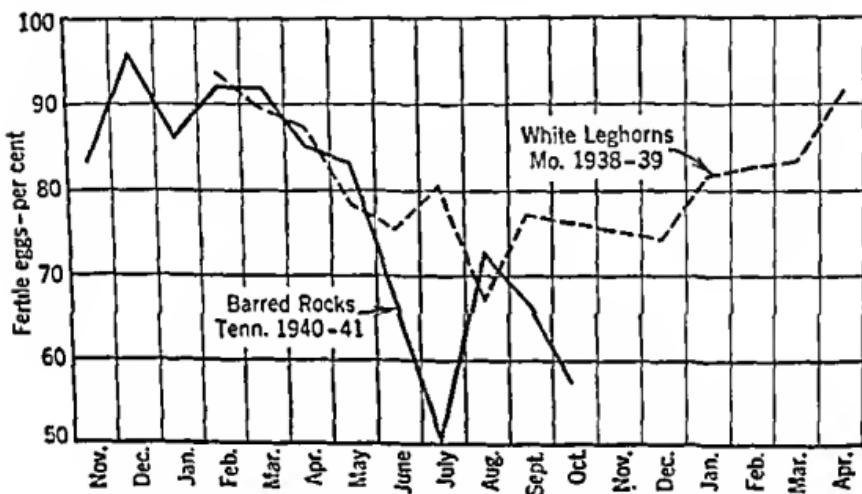


FIG. 31. Seasonal variation in fertility of chickens. Courtesy Tennessee Agr. Expt. Sta.

sistently until the outside temperature was about 37° F. When mean weekly outside temperatures ranged from 23° to 50° F., Lamoreux observed no relationship between the temperature and the fertility of White Leghorns. He indicated that low temperature is unlikely to have any effect on fertility as long as combs and wattles are not frozen. In connection with the two experiments mentioned above, which are not in agreement, it should be pointed out that the White Leghorns were exposed to artificial light whereas the Rhode Island Reds were not. It is conceivable that the effect of artificial light on the birds may have accounted for the differences in the results.

Whether fertility in the domestic fowl is inherited is disputed. Many of the earlier investigations conducted to answer the question gave negative results. Pearl and Surface (1909) and Hays and Sanborn (1924; 1939) found no evidence that fertility was

hereditary, but Jull (1935) reported a low but significant correlation between the fertility of dams and that of their daughters. More recently Blyth (1945) concluded that fertility is an inherited characteristic. As pointed out previously, it has been demonstrated that the age at which cockerels attain sexual maturity is influenced by heredity (pp. 115-116).

Inbreeding has been shown to decrease fertility by Dryden (1918), Hays (1924; 1929), Jull (1930; 1933), and Knox (1946). On the other hand, Waters and Lambert (1936) found no significant differences in fertility between progeny having different degrees of inbreeding and concluded that inbreeding had no serious effect on fertility probably because of very severe incidental selection for this character. Jull (1933) reported that the crossing of inbred lines resulted in lower fertility than was found in the inbred matings of the previous year; this was a surprising result. More recent studies by Bernier (1947) revealed that inbreeding, outcrossing, crossbreeding and inbreeding, to varying degrees, apparently do not affect fertility directly—or, in other words, fertilization of the egg is neither impaired nor enhanced by the presence of sperms from a related or an unrelated male. Bernier concluded that fertility is a property of the parents and not of the prospective zygotes resulting from the mating. Two lines of females with inbred origins showed a higher incidence of infertile matings and were also less fertile than females with an outbred origin. It has also been shown by Knox (1939) that crossbreeding had no effect on fertility.

Hutt (1940) cited data indicating that fertility in White Wyandottes was lower than in some other breeds, possibly because of a hereditary weakness for this characteristic. Munro (1946) tended to minimize the influence of inheritance on the fertility of Wyandottes and believed that environmental factors probably were more important.

Social dominance or "peck order" has been shown to be related to the sexual activity and fertility of chickens. Literature in the field has been reviewed by Guhl, Collias, and Allee (1945) and by Guhl and Warren (1946). The former group showed that hens with high social positions were not mated as often by the males as hens with lower peck orders. On the other hand, both group-

of workers showed that dominant males mated more frequently and fertilized more eggs than males that were dominated.

FERTILITY IN TURKEYS

Fertility in the Male Turkey

In most respects the reproductive system of the male turkey is similar to that of the male chicken; there is a slight difference, however, in the shape of the copulatory organ (fig. 21). In the cock the organ terminates in a single apex, whereas in the turkey tom the organ has a double apex (Burrows and Quinn, 1937). Harper and Parker (1947) observed that at the end of the breeding season both gonads of Broad Breasted Bronze toms weighed only 25 grams, which is no greater than, if as great as, weights reported for chicken testes.

The development of fertility in male turkeys is of considerable interest and importance to turkey breeders. Semen containing active spermatozoa was collected from Broad Breasted Bronze turkeys by Parker (1946a) in mid-December when the toms were 7 months old and had not been exposed to artificial lighting. Lorenz and Lerner (1946) found that the range in age at sexual maturity, as measured by age of first appearance of semen, was 137 to 293 days with an average of 228 days. Studies reported by Margolf, Harper, and Callenbach (1947) showed that White Holland turkeys matured at approximately 7 months and that spermatogenesis, if not already present, could be induced at this age by suitable exposure to light. They reported that complete spermatogenesis is attained in male turkeys at the end of the normal growth period, after which the gonads regress toward a quiescent state. There was some indication that the longer the toms remained in this sexually quiescent state, the greater was the amount of lighting required to reactivate them. Approximately 5 weeks of lighting was required for the resting testes to come into full sperm production (fig. 32). It was also shown by Margolf *et al.* that the most active mating period preceded the onset of egg production and that for this reason the toms should be subjected to lighting before the hens. Milby and Thompson (1945), on the other hand, reported data indicating

that toms do not need to be exposed to artificial light before the hens.

Amounts of semen collected from individual turkey toms by Burrows and Marsden (1938) varied from 0.1 to 0.7 cubic centi-

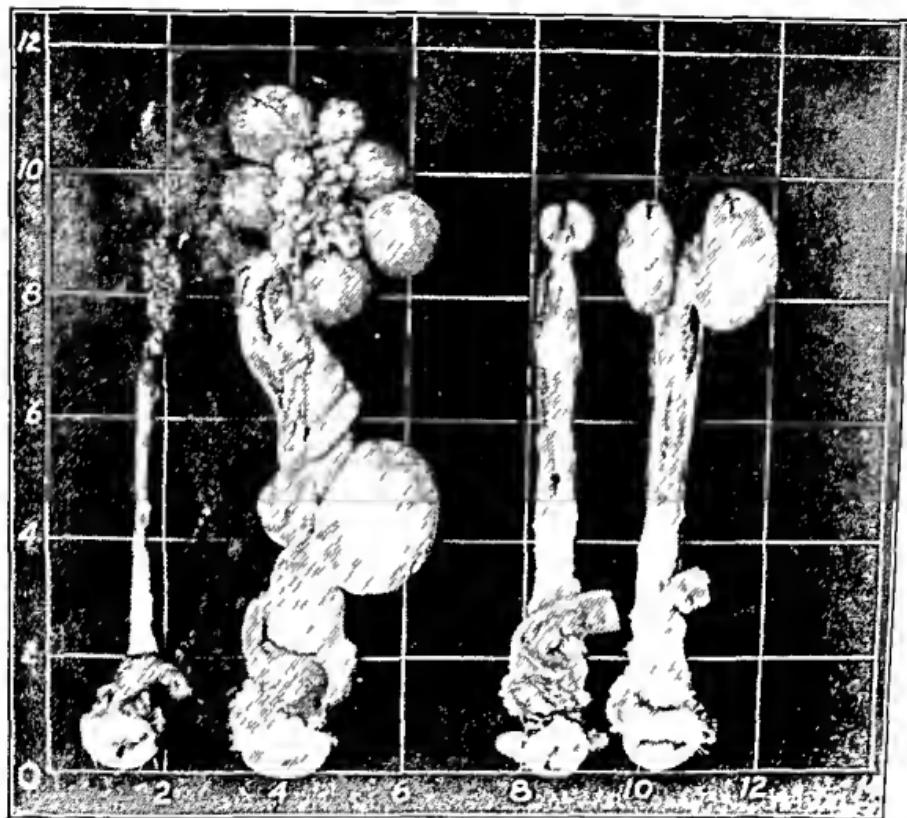


FIG. 32. Reproductive systems of female and male turkeys exposed to no artificial lighting (left specimen of each pair) and to 5 weeks of lighting (right specimen of each pair). *Courtesy Pennsylvania Agr. Expt. Sta*

meter, the most common volumes being 0.3 to 0.4 cubic centimeter. Parker (1946a) collected similar semen volumes from Broad Breasted Bronze toms; he reported the average volume to be 0.33 cubic centimeter; the average density, 8.4 million sperms per cubic millimeter of semen; and the average number of sperms per ejaculate, 2.8 billions. Existing data (table 3) indicate that turkeys produce about half the volume of semen produced by chickens but that the sperm concentration in turkey semen is

aging 92 per cent fertile, whereas eggs from nonbroody hens were 82 per cent fertile. It should be pointed out, however, that in spite of their lower fertility the nonbroodies produced more pouls per hen because of higher egg production. Margolf, Harper, and Callenbach (1947) noticed increased mating activity during

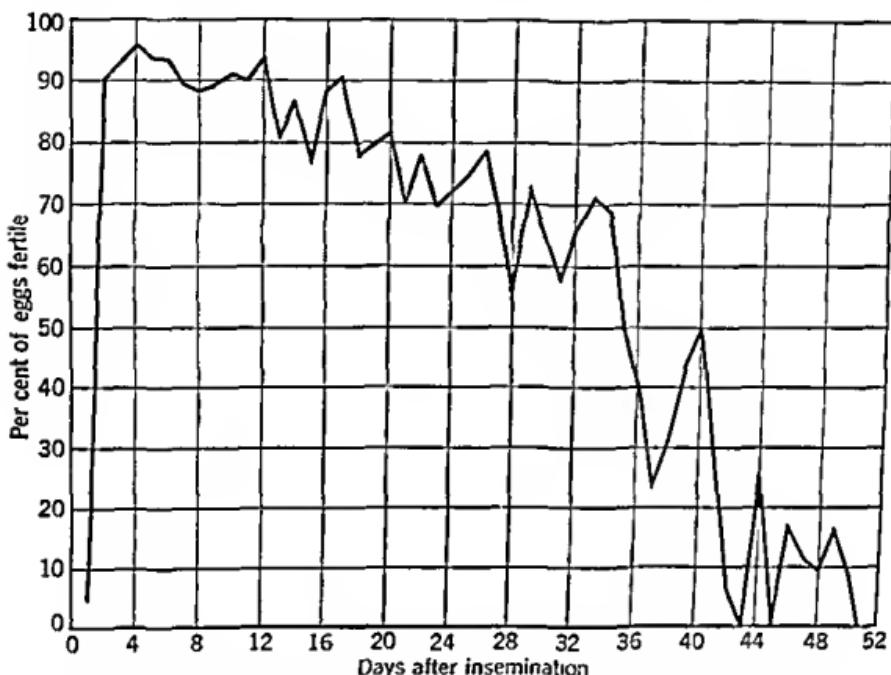


FIG. 33. Duration of fertility in turkey hens after one insemination with 0.05 cubic centimeter of semen. A total of 2103 eggs from 61 hens was involved. *Courtesy F. W. Lorenz, Univ. California.*

periods of increased broodiness which was due in large measure to resumed mating by broken-up broody hens. More recently Jones and Kohlmeyer (1947) reported that turkey hens with either pause or broody periods were no more fertile than continuous layers.

Although the afternoon insemination of chicken hens is conducive to highest subsequent fertility, it has not been shown that the time of mating affects fertility in turkeys. Limited data on the subject by Parker and Barton (1946) showed that the difference in fertility of eggs from hens artificially inseminated in the morning and in the afternoon was too small to be significant.

not affected, but that such exposure for 48 hours reduced hatchability considerably, and that 72 hours of such refrigeration destroyed the hatching power of the eggs.

It was reported by Mussehl and Bancroft (1924-1925) that the hatchability of chicken eggs was not lowered when the eggs were

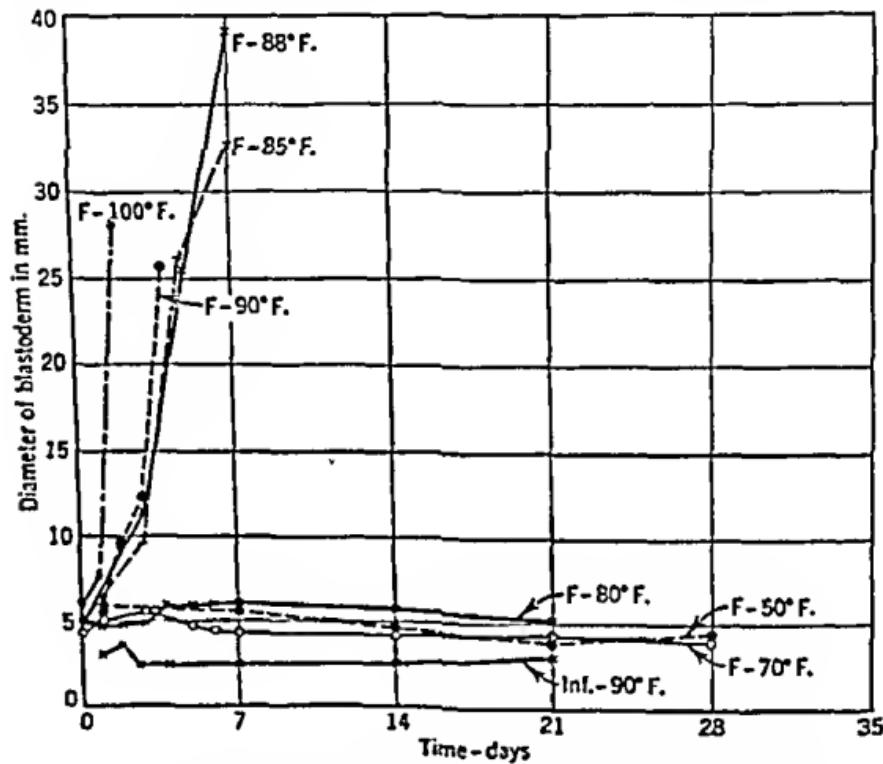


FIG. 40. Effect of temperature on the development of the blastoderm of fertile (F) eggs. Courtesy Missouri Agr. Expt. Sta.

TABLE 8

EFFECT OF CHILLING HATCHING EGGS ON THEIR HATCHABILITY
 (Mussehl and Bancroft, 1924-1925)

Treatment	Eggs set	Percentage hatch	
		All eggs	Fertile eggs
<i>Series 1 (Exposed to 32° F.)</i>			
Eggs not chilled	465	50.9	63.2
Eggs chilled for 6 hours	463	54.9	65.5
<i>Series 2 (Exposed to 15° F.)</i>			
Eggs not chilled	200	59.5	64.3
Eggs chilled for $\frac{1}{2}$ hour	200	70.0	73.6
Eggs chilled for 1 hour	200	66.5	70.7
Eggs chilled for $1\frac{1}{2}$ hours	200	66.0	71.7
<i>Series 3 (Exposed to 32° F.)</i>			
Eggs not chilled	179	54.7	63.2
Eggs chilled 6 hours	179	66.4	71.6
Eggs chilled 12 hours	176	56.8	65.7
Eggs chilled 18 hours	173	56.6	67.1

to hatch. These results indicate that a critical point exists between the sixth and eighth day of exposure to temperatures slightly above freezing.

Table 9 gives the results of some experiments, reported by Funk (1934), in which eggs laid by White Leghorns were exposed

TABLE 9

EFFECT OF LOW TEMPERATURES BEFORE INCUBATION ON HATCHABILITY
 (Funk, 1934)

Time held at 32° F. to 38° F. (hours)	Eggs set	Percent- age of infertile eggs	Percent- age of dead embryos	Chicks hatched	Percentage hatch	
					All eggs	Fertile eggs
6	194	9.8	21.6	133	68.6	76.0
12	131	11.5	20.6	89	67.9	76.7
48	105	9.5	17.1	77	73.4	81.1
96	193	31.1	23.8	87	45.1	65.4
120	58	12.1	51.7	21	36.2	41.2
144	78	48.7	48.7	2	2.6	5.0
168	59	35.6	64.4	0	0.0	0.0
192	63	31.7	68.3	0	0.0	0.0
Controls	732	9.0	20.1	533	70.9	77.9

to relatively low temperatures for 6 to 192 hours. These data show that eggs laid by this strain of White Leghorns were held for 48 hours at temperatures of 32° F. to 38° F. without detrimental effect upon hatchability. However, when the eggs were held at these temperatures for 96 hours, hatchability was lowered considerably, and after 7 days of such exposure it was reduced to zero.

Funk also collected hatching records from hatcheries situated in Missouri and reported upon the relationship (fig. 41) of weather conditions to hatching results. He found that the percentage hatch of all eggs set was generally reduced by 10 per cent or more during cold waves. The reasons for this reduction may be attributed to (1) the excessive chilling of hatching eggs, (2) the inactivation of males by reason of frozen combs and wattles, and, possibly, (3) the effect of cold upon the metabolism of the breeding birds.

Phillips (1945) reported the results of experiments made at the Maryland Agricultural Experiment Station with New Hampshire eggs held for 1 to 7 days at 32° F., 38° F., and 52° F. His data (table 10) show that neither age nor temperature had a consistent effect on hatchability within the limits of his experiments.

TABLE 10
EFFECT OF HOLDING TEMPERATURES ON HATCHABILITY
(Phillips, 1945)

Setting	32° F.		38° F.		52° F.	
	No. fertile eggs	Percentage hatch- ability	No. fertile eggs	Percentage hatch- ability	No. fertile eggs	Percentage hatch- ability
1	254	87.2	258	81.7	250	84.0
2	279	84.2	281	82.9	275	86.5
3	302	80.1	295	80.3	302	81.8
4	286	85.2	289	85.4	291	84.6
5	355	88.4	301	87.3	354	86.0
All	1476	85.1	1421	83.6	1472	81.6

The lack of agreement among the data reported by the different investigators is notable and requires some explanation. All these results are no doubt correct for the exact conditions under which

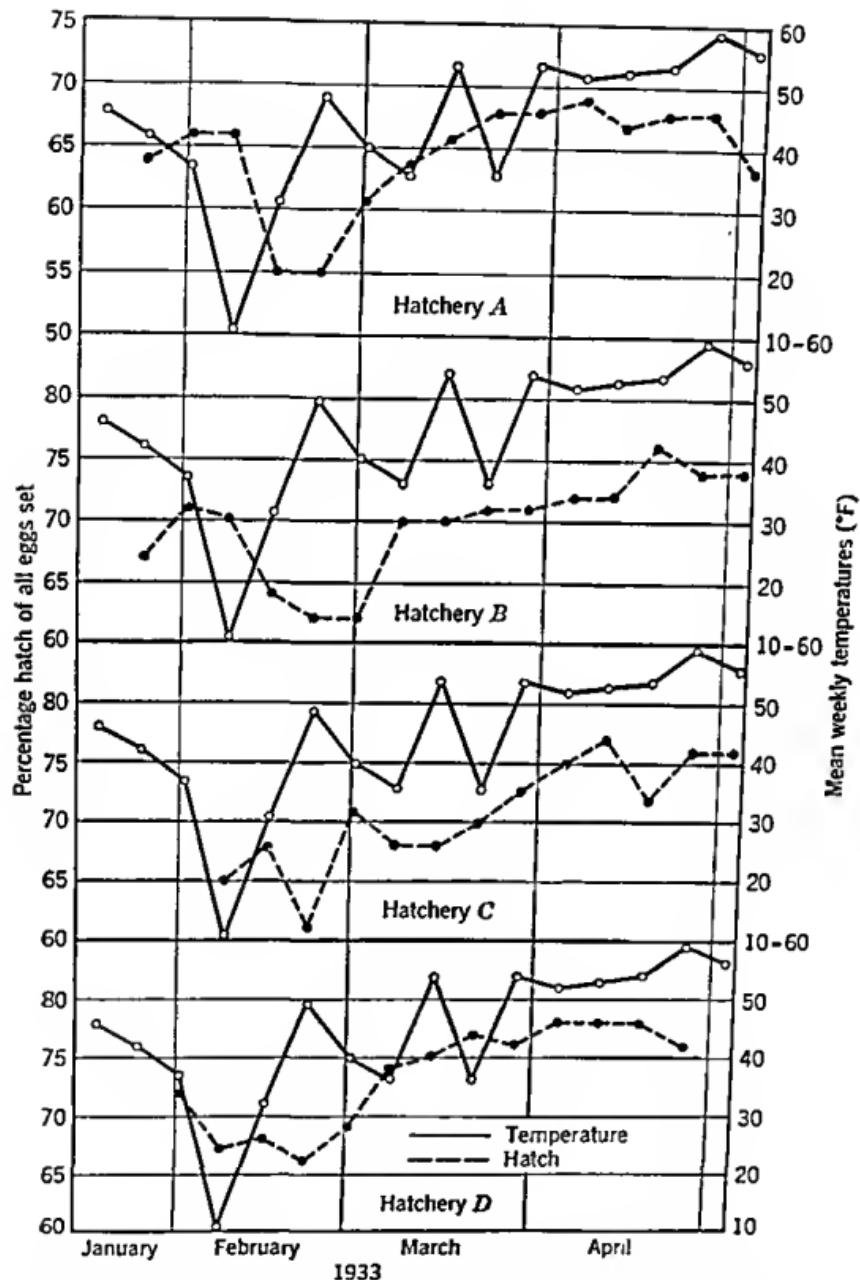


FIG. 41. Effect of a sudden drop in temperature (weather station reports) on hatching results. Courtesy Missouri Agr. Expt. Sta.

they were obtained. In some experiments, however, temperatures varied considerably, whereas in others they were carefully regulated. The strains and breeds of the chickens whose eggs were used also varied; Phillips was working with a breed (New Hampshire) which produces eggs that may possibly be more resistant to cold than the Leghorn eggs used by Scott and by Funk.

Effect of Subfreezing Temperatures

It is not uncommon during cold weather for hatcherymen to find entire trays in which few or no eggs hatch. The fact that when candled such eggs appear clear or apparently infertile indicates that the embryos were killed by chilling. Completely normal 8-day embryos were reported by Colasanti (1875) from eggs that had been submerged in concentrated salt solution with ice for 2 hours; he reported that the eggs were completely frozen with internal temperatures of -7° to -10° C. (19.4° to 14° F.). Elford (1921) reported 80 per cent hatchability from eggs packed and held for 5 hours at 14° F. to 26° F. Grodzinski (1933) held freshly laid eggs for 4 days at -3° C. (26.6° F.), and these eggs, when incubated, failed to develop embryos or blood vessels; there was an enlargement of the blastoderm but only ectoderm and entoderm developed. Such eggs if examined by candling would appear clear or infertile.

Jull, McCartney, and El-Ibiary (1947) reported that holding chicken (New Hampshire) hatching eggs at -1° F. for as long as 10 hours and with a reduction of the internal egg temperature to 30.2° F. did not seriously impair their hatchability. Turkey eggs exposed to -1° F. for 1, 2, 3, or 4 hours hatched more poult than did control eggs held at 50° F. to 55° F. Jull *et al.* further reported hatching some chicks from eggs in which the temperature had been reduced to 3.2° F.

Effect of High Temperatures on the Hatchability of Eggs

As early as 1909, Philips reported that eggs held for 14 days at 80° F. failed to hatch. Funk (1931) attempted to determine the effects of high environmental temperatures on the hatchability of chicken eggs. He held eggs at incubation temperature (101° F.) in a sectional incubator for several hours immediately after the eggs were laid and before they cooled. These data (table 11),

TABLE 11

EFFECT OF HOLDING EGGS AT INCUBATION TEMPERATURE IMMEDIATELY AFTER OVIPOSITION
 (Funk, 1934)

Hours of exposure to 101° F.	Eggs set	Percentage hatch of all eggs
0	85	65
6-8	88	73
12-14	89	69
18-20	84	46
24-26	82	48

though not based on large numbers, show that eggs incubated for 18 to 20 hours or longer and then cooled lost much of their hatching power. Temperatures above 80° F., at which growth is initiated, would also produce this result because there would be rapid embryonic development at 85° F. to 100° F.

In the same paper Funk presents a report on the experience of Missouri hatcherymen during the summer months (fig. 42). Whenever the mean (average) weekly temperature was above 80° F. the percentage hatch of all eggs set declined 15 to 20 per cent. This decline in hatching performance may perhaps be attributed to two conditions: (1) preincubation development and weakening of the embryos before the eggs were received at the hatchery and (2) the decreased fertility which is associated with inactive breeding stock in hot weather. Hatches became normal with the return of cooler weather in the fall.

Talmadge (1947) reported hatching results when White Plymouth Rock eggs were held for 1 to 7 days at 90° F., at 38° F. to 40° F., and at 70° F. to 76° F. He concluded that holding eggs for 1 to 7 days at 38° F. to 40° F. and at 70° F. to 76° F. had no detrimental effect on hatchability. He reported further that the hatchability of eggs stored at 90° F. for 1 to 4 days was not affected, but that eggs held at this temperature for 5, 6, or 7 days hatched very poorly.

Devitalizing Fertile Eggs

The results reported by a number of investigators, as discussed in the section on the effects of low temperatures, show that chil-

ing devitalizes an egg if the eggs are exposed to freezing (32° F.) for several days. Eggs held long enough at any temperature other than the normal incubation temperature also lose their power of

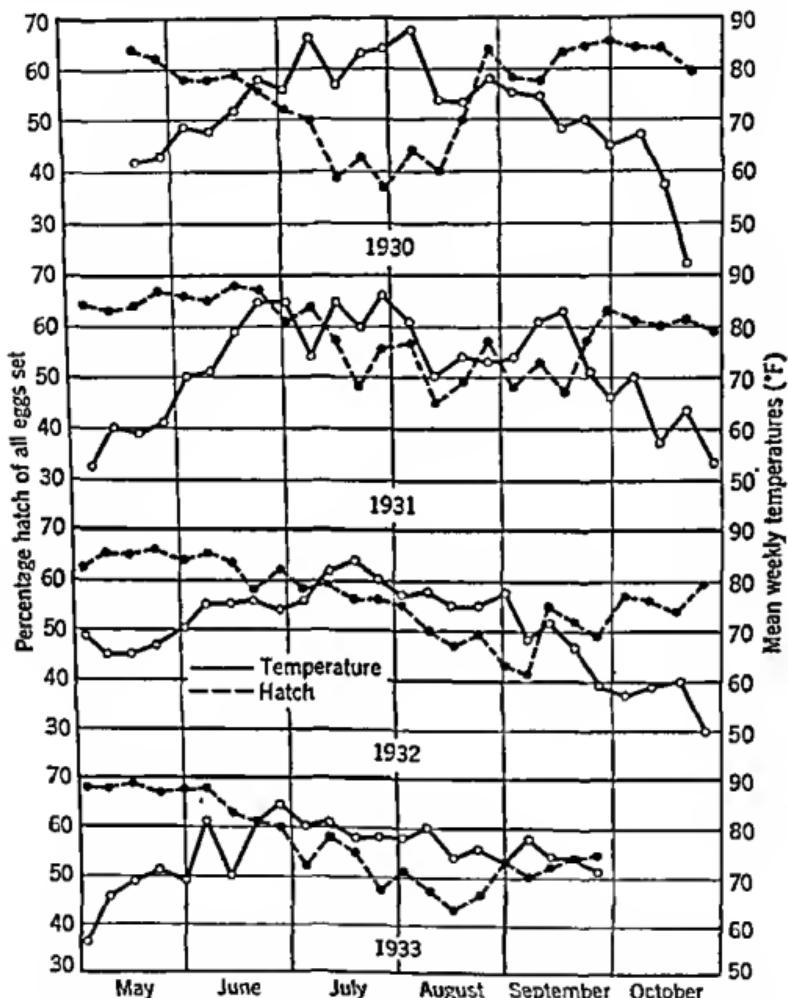


FIG. 42. Effect of summer temperatures on hatching results. Courtesy Missouri Agr. Expt. Sta.

embryonic development. In 1943, Funk reported results of experiments in which fertile eggs were devitalized by immersion in water for 35 minutes at 120° F., for 15 minutes at 130° F., for 10 minutes at 138° F., and for 5 minutes at 140° F. This rapid process for devitalizing fertile eggs was developed in order to

eliminate the fertility factor as a cause of deterioration of quality in market eggs. Also the process has practical application for anyone who desires to destroy the hatching power of eggs without impairing their market quality; for example, it is conceivable that specialty breeders may wish to render incapable of embryonic development such eggs from their stock as may pass into the regular market channels for food.

Optimum Temperature for Holding Hatching Eggs

Investigators and hatcherymen have been primarily interested in finding the ideal temperatures at which hatching eggs may be held. Scott (1933) reported several series of experiments with both chicken and turkey eggs in which he held the eggs at different temperatures for as long as 34 days. His data show that Leghorn eggs that had been held at 54.2° F. for as long as 20 days hatched nearly as well as eggs less than 7 days old (table 12). This result may be due to the maintenance of a relatively

TABLE 12

EFFECT OF AGE OF EGG AND HOLDING TEMPERATURES ON THE
HATCHABILITY OF CHICKEN EGGS
(Scott, 1933)

No. of days	Percentage hatchability at	
	36.3 ± 0.2° F.	54.2 ± 0.26° F.
0-6	63.3	69.4
7-13	5.3	66.7
14-20	0.0	67.9
21-27	0.0	44.3
28-34	0.0	32.1

constant temperature. Another interesting observation is that this temperature must be near the optimum.

Scott's results with turkey eggs have been summarized in table 13. It is evident that temperatures above 60° F. were detrimental to the hatchability of turkey eggs when the eggs were held at such temperatures for more than 14 days. It is also apparent that when turkey eggs were held at 36° F. for even as short a period as 6 days their hatchability was lowered. With temperatures of 55° F. to 60° F. excellent hatchability was obtained, and a constant temperature of 54° F. gave good results. These re-

TABLE 13

EFFECT OF AGE OF EGG AND HOLDING TEMPERATURES ON THE
HATCHABILITY OF TURKEY EGGS
(Scott, 1933)

No. of days	Percentage hatchability			
	(1929) 60-75° F.	(1930) 55-60° F.	(1931) 36.3 ± 0.2 ° F.	(1931) $54.2 \pm .26$ ° F.
0-6	71.9	89.3	65.6	71.0
7-13	73.4	89.7	52.4	65.0
14-20	44.6	84.8	26.8	74.6
21-27	14.1	84.0	5.9	67.3
28-34	6.3	85.7	0.0	61.1

sults, however, are not in agreement with other work with turkey eggs; Asmundson (1947), for example, reported that turkey eggs held for 7 days or longer at 55° F. showed a gradual decrease in hatchability of nearly 10 per cent each week as compared to turkey eggs from the same flock held for 0 and 3 days. More work is needed on this problem.

Funk (1934) reported hatching results with chicken eggs held in a basement room where the temperature varied from 45° F. to 60° F. These results showed that hatching eggs held for longer than 14 days at 45° F. to 60° F. gradually lost their hatching power until, at the end of 28 days, hatchability had been reduced to zero (table 14).

TABLE 14

EFFECT OF AGE OF EGG UPON HATCHABILITY WHEN CHICKEN EGGS WERE HELD IN A BASEMENT ROOM VARYING FROM 45° TO 60° F.
IN TEMPERATURE
(Funk, 1934)

No. of days	Percentage hatchability
1-7	76.2
8-14	74.0
15-21	64.6
22-28	32.0
29-31	0.0

Warren (1934) reported the results of holding eggs laid by White Leghorns and Rhode Island Reds in a room where the

temperature varied from 55° F. to 65° F. except on 1 day a week when the temperature was raised to 75° F. for about 4 hours. Under those conditions hatchability was impaired after 6 days.

Heywang (1945) published data on an experiment conducted in Arizona to determine the value of frequent gathering and cooling of hatching eggs laid during hot weather (table 15). The

TABLE 15

EFFECT OF TIME OF GATHERING, PREVAILING OUTSIDE AIR TEMPERATURES,
AND STORAGE TEMPERATURES ON THE HATCHABILITY OF EGGS
(Heywang, 1945)

Method of gathering and storing eggs	Preliminary period * percentage hatchability	Percentage hatchability at		
		Average max. outside air temp.		
		90-95	100-105	105-109
Storage temp.				
		55°	55°	55°
(1) Placed in refrigerator immediately after laying	75.6	77.6	72.3	49.3
(2) Gathered at noon and 5 p.m. and placed in refrigerator	76.0	69.9	68.0	39.2
(3) Gathered at 5 p.m. and placed in refrigerator	77.2	77.7	71.4	53.3
Storage temp.				
		76°	80°	86°
(4) Gathered at noon and 5 p.m. and placed in cellar	73.8	65.4	39.1	26.7
(5) Gathered at 5 p.m. and placed in cellar	79.5	61.5	40.6	28.4

* Cool weather prevailed during this period.

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(3) Gathered at 5 p.m. and placed in refrigerator	77.2	77.7	71.4	53.3
Storage temp.				
		76°	80°	86°
(4) Gathered at noon and 5 p.m. and placed in cellar	73.8	65.4	39.1	26.7
(5) Gathered at 5 p.m. and placed in cellar	79.5	61.5	40.6	28.4

* Cool weather prevailed during this period.

eggs were removed from the trap nests at hourly intervals; they were not held more than 7 days. From his results it is evident that eggs stored at 76° F., 80° F., and 86° F. lost much of their hatching power within 7 days. These data also indicate that when hatching eggs were gathered once (5 P.M.) or twice daily (noon and 5 P.M.) hatchability was not reduced below that of eggs gathered and stored hourly at 55° F. Eggs produced during extremely hot weather (105° F.-109° F.) hatched very poorly (26.7 to 53.3 per cent of the fertile eggs). Even though the eggs were gathered hourly during the hot weather and stored at 55° F., they gave a poor hatch (49.3 per cent).

THE AGE OF THE EGG

The hatchability of any fertile egg is destroyed when it is held for an extended period at temperatures other than normal incubation temperature. The length of time a hatching egg may be held without impairment of its hatching quality depends very largely upon the temperature at which the egg is held.

Waite (1919) reported the results of a study of the effect of the age of eggs on their hatchability. This report covered the period from 1914 to 1919 inclusive and included 189 incubator records on 26,415 eggs. The eggs were held "on trays in a basement room at cellar temperature." This was a comprehensive study under the conditions that prevailed at that time, but it should be noted that temperature was not recorded. The temperature was, no doubt, variable and not optimum. Waite prepared the chart given in figure 43 which showed that the percentage of hatch declined steadily after the eggs were held for 5 or 6 days. Fifty-six eggs held for 28 days produced only three chicks. Eggs held under general farm conditions are probably affected by age (the time held), as Waite found them to be in 1919.

Scott (1933), as previously stated, showed that chicken eggs held for as long as 20 days at a constant temperature of 54° F. hatched as well as eggs held less than 1 week at the same temperature. The difference between his results and those of Waite can probably be explained by the difference in temperature.

From the work that has been done it is evident that hatchability may be completely destroyed at subfreezing temperatures

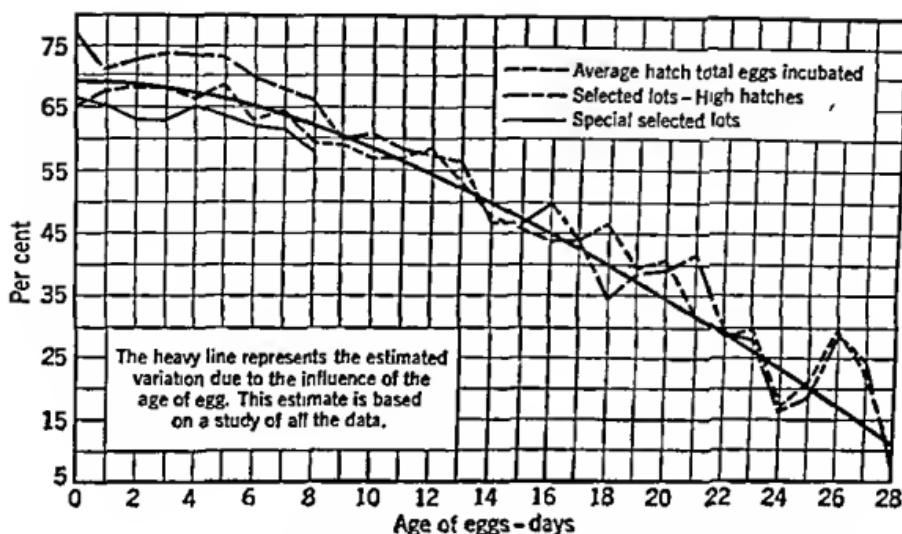


FIG. 43. Effect of the age of eggs on their hatchability. Courtesy Maryland Agr. Expt. Sta.

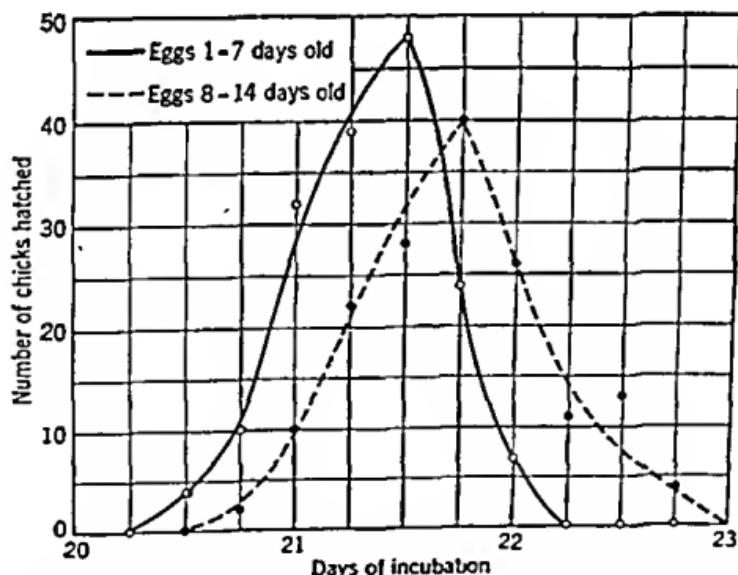


FIG. 41. Effect of the age of eggs on the length of the incubation period. Courtesy Missouri Agr. Expt. Sta.

and at high temperatures (above normal incubation temperatures). At constant and optimum temperatures eggs may be held for as long as 20 days without impairment of their hatchability. However, under the conditions that generally prevail hatching eggs should be delivered to the hatchery at least once each week.

Asmundson (1946), in reporting the results of holding turkey eggs, summarized:

The eggs held for 7 or more days did not hatch so well as those set within 1 to 4 days of the time they were laid. There was a steady decrease of 6 to 9 per cent each week to 28 days with a sharp reduction after that date.

Holding the eggs for 2 days at 70° F. apparently had little or no effect on hatchability.

There is an indication that cooling eggs at about 30° F. for not over 6½ hours improved hatchability. There is also a slight indication that cooling eggs for 24 hours at 40° F. increases hatchability. The data suggest that hatchability is reduced if eggs are held too long at such temperatures, but the data on temperature effects cannot be considered conclusive.

Funk (1934) and Kaufman (1938) have shown that older chicken eggs require more time to complete incubation and hatch than eggs held for shorter periods (fig. 44). However, Olsen (1942) found that when turkey eggs were held from 1 to 16 days the length of the incubation period was not related to the age of the eggs.

THE EFFECT OF HUMIDITY

The relative humidity of the atmosphere in which hatching eggs are held may have an effect on hatchability. Such an effect may be expected if extremely high or extremely low humidity prevailed. However, it has not been determined that hatchability is affected by the humidities which generally prevail where hatching eggs are held. North (1941) found that in Wyoming, when hatching eggs were held 10 days or longer, hatchability was increased by 4 per cent when the eggs were held where the relative humidity was 50 per cent as compared to the hatchability of eggs held at a lower relative humidity.

From the results of an experiment at the Oregon Agricultural Experiment Station, Cooney (1943) concluded that eggs held for

4 to 14 days where the relative humidity was approximately 90 per cent hatched about 5 per cent more chicks than eggs held where the relative humidity varied from about 60 to 85 per cent.

These results suggest the need for a more detailed study of this problem under carefully controlled conditions of temperature and humidity.

TURNING HATCHING EGGS BEFORE INCUBATION BEGINS

Should hatching eggs be turned between the time they are laid and the time they are set? This question has not been answered to the satisfaction of everyone. Jackson (1912) reported that the turning of hatching eggs did not improve their hatchability. From results presented in table 16 and other data Waite (1919)

TABLE 16

EFFECT ON HATCHABILITY OF TURNING HATCHING EGGS DURING THE HOLDING PERIOD
(Waite, 1919)

Age of eggs (days)	Percentage hatch of all eggs set	
	Eggs turned daily	Eggs not turned
0-10	44.52	39.55
11-20	34.37	35.07
21-28	13.63	18.18

also concluded that the turning of hatching eggs before incubation was unnecessary if not actually harmful to hatchability.

Dougherty (1928), on the other hand, reported results from an experiment in which he attempted to measure the value of turning hatching eggs held in different positions (table 17). The number of eggs used in this experiment is not given but it appears that, under the conditions which prevailed in this experiment, turning did improve hatchability.

Funk (1934) conducted an experiment covering 9 weekly hatches during the 1934 hatching season with eggs held for 1 to 7 days in a basement room where the temperature varied from 45° F. to 60° F. His data, as presented in table 18, show that under the conditions of this experiment daily turning was of no

TABLE 17

EFFECT ON HATCHABILITY OF TURNING HATCHING EGGS HELD ON THEIR SIDES AND ON THEIR ENDS IN CASES

(Dougherty, 1928)

Age of eggs (days)	Percentage hatchability of eggs			
	Held in racks		Held in cases	
	Turned daily	Not turned	Turned daily	Not turned
6	76	70	80	58
10	83	70	73	66
14	74	60	72	55

TABLE 18

EFFECT ON HATCHABILITY OF TURNING EGGS BEFORE INCUBATION

(Eggs held for 1 to 7 days before being incubated.)

(Funk, 1934)

Hatch	Turned		Not turned	
	Eggs set	Percentage hatch of fertile eggs	Eggs set	Percentage hatch of fertile eggs
2	202	68.4	301	70.6
3	266	68.3	298	70.9
4	222	60.5	257	60.9
5	219	69.1	281	76.7
6	368	71.9	442	73.8
7	232	71.2	270	78.6
8	216	80.6	308	79.8
9	213	61.7	282	73.1
10	193	65.2	205	68.1
All	2161	69.3	2644	72.6

value for hatching eggs held for 1 to 7 days. In fact, the percentage of hatch was higher in 8 of the 9 hatches when the eggs were not turned. If such results could be substantiated by experiments with larger numbers of eggs, Waite's conclusion that turning may be harmful might be verified.

One reason commonly given for turning hatching eggs before incubation is that such turning will prevent the blastoderm from sticking to the shell membranes. The author has found, however, that normally the blastoderm does not stick to the shell membranes until age has destroyed the hatching power of the egg.

THE HANDLING OF HATCHING EGGS

When the shell of an egg is cracked by rough handling the hatchability of that egg is usually destroyed. However, each year millions of hatching eggs are shipped hundreds of miles by truck or rail without serious impairment of their hatching power; in fact, shipped eggs sometimes hatch better than eggs produced locally. Nevertheless, it is generally true that the hatchability of eggs is lowered by shipping.

Dareste (1885) reported that in a high percentage of eggs that were jarred abnormal embryos developed when the eggs were held with the small end up but that relatively few abnormal embryos resulted when eggs were jarred when held with the small end down. The mechanical shaking of hatching eggs was reported by Landauer and Baumann (1943) to increase very markedly the incidence of rumplessness in the strain of White Leghorns used. More rumplessness occurred when eggs were shaken with the large end down than when they were shaken with the large end uppermost. When the eggs were allowed to rest for 24 or 48 hours after the shaking, the incidence of rumplessness was reduced. Landauer and Baumann also found that the occurrence of other abnormalities was increased by shaking the eggs. Knox and Olsen (1936) reported results of experiments in which hatching eggs were jarred. When the eggs were jarred with the small end down hatchability (60.8 per cent) was not reduced below that (60.8 per cent) of the eggs which were not jarred, as compared to a much lower hatch (36.9 per cent) of eggs jarred with the large end of the eggs down. They

produced tremulous air cells in freshly laid eggs by jarring eggs held with the large end down. The hatchability (56.5 per cent) of these eggs was 20.8 per cent below that of the eggs which were not jarred and which therefore did not have tremulous air cells. It is evident from these results that eggs jarred sufficiently to cause tremulous air cells do not hatch well.

Knox and Olsen (1934) showed that eggs packed in regular fillers on cup flats developed a higher percentage of tremulous air cells when shipped than did eggs packed in more rigid packs such as filler-flats, or in regular fillers with sawdust filling the spaces, or with paper wrapped around the eggs before they were placed in the fillers. Since tremulous air cells and poor hatchability are related, rigid packs in shipping hatching eggs would probably improve hatchability sufficiently to warrant their more general use. Possibly a more practical type of filler and flat is needed, one that will be more generally accepted by the trade.

Tremulous air cells can readily be detected by candling, and therefore eggs showing this defect can be eliminated from hatching eggs before they are set. The work of Knox and Olsen also indicates the practical importance of placing eggs in cases with the small end down; if all eggs were placed in this position before handling, hatchability would be appreciably increased.

THE EFFECTS OF SEALING THE EGG SHELL

Since the developing embryo in an egg must receive its oxygen from the air which passes through the pores of the egg shell, the closing of these pores by sealing with any substance will cause the death of the embryo. Two centuries ago, de Réaumur (1751) showed that eggs made impermeable to air would not hatch, and Theodor Schwann (1834) later proved that air was essential for embryonic development. Gerlach (1880) and Gerlach and Koch (1883) showed that when part of the surface of eggs was varnished many of the embryos which later developed were dwarfed or otherwise abnormal. Düsing (1884) reported that one-half of the shell surface of eggs could be sealed without ill effects but that the sealing of 65 to 70 per cent of the shell surface increased the number of abnormal embryos.

Byerly and Olsen (1931) found that as a result of the coating of the large end of an egg with paraffin the proportion of chicks in normal hatching position (i.e., with the head under the right wing and in the large end of the egg) was reduced from 91.7 per cent to 48.9 per cent and that the number of chicks developing with the head in the small end of the egg was increased from 1.3 per cent to 27.3 per cent. They also showed that only 14.8 per cent of the chicks that developed with the head in the small end of the egg were able to hatch.

Hall and Romanoff published in 1943 the results of their experiments designed to determine the effect on the subsequent hatchability of eggs of sealing the shell while the eggs were held previous to incubation. They held eggs submerged in distilled water, gelatin solution, and sodium silicate solution. Their data with eggs held at 50° F. are given in table 19. Hatchability was

TABLE 19

EFFECT UPON HATCHABILITY OF HOLDING EGGS IN VARIOUS LIQUIDS
(Hall and Romanoff, 1943)

Holding conditions	Hatchability (per cent) after holding			
	1 week	2 weeks	3 weeks	4 weeks
In air	77.8	78.9	41.2	33.3
Submerged in distilled water	64.7	35.9	6.3	0.0
Submerged in gelatin solution	41.2	0.0	0.0	0.0
Submerged in sodium silicate solution	0.0			

found to be reduced when eggs were held submerged in liquids. The cause of this reduction in hatchability was not determined.

CLEANING SOILED HATCHING EGGS

Most soiled eggs occur during the spring months when most hatching eggs are produced. Can these eggs be cleaned without impairing their hatchability? If they are valuable chicken or turkey eggs they are worth saving. Dry cleaning requires considerable labor, and the merits of wet cleaning have not been

thoroughly investigated. In some unpublished preliminary tests, the writer found (table 20) that the hatchability of eggs that were coated with whole egg material was very low, 34.2 per cent as compared to 84.1 per cent for clean eggs. This experiment also showed that eggs that were coated with liquid whole egg when cleaned by washing with water or with 1 per cent lye water hatched about as well as clean eggs. The average percentage

TABLE 20

EFFECT OF CLEANING EGGS ON HATCHABILITY
(Funk, 1939)

Treatment	No. eggs set	Percentage hatch	
		All eggs	Fertile eggs
Clean eggs for controls	196	75.5	84.1
Clean eggs dipped in liquid whole egg	201	31.8	34.2
Clean eggs dipped in liquid whole egg and after drying cleaned by washing with water	201	80.1	84.3
Clean eggs dipped in liquid whole egg and after drying washed in 1 per cent lye water solution	196	65.8	72.1

hatch of all eggs for the two washed lots combined was 72.9 per cent as compared to 75.5 per cent for the controls. Since these tests were limited in the numbers of eggs used, additional experiments are needed to clarify the effect of these methods of cleaning soiled eggs.

Pritsker (1941) reported the results of Russian investigations in which dirty eggs were washed under increasing or decreasing internal pressures. These internal pressures were achieved by immersing the eggs in solutions either colder or warmer than the eggs (table 21). The superiority of washing with warm solutions was clearly indicated. Pritsker also conducted experiments in disinfecting hatching eggs with a 0.5 per cent solution of formalin with some eggs under increasing and others under decreasing internal pressures. Again better results were obtained with solu-

TABLE 21

EFFECT OF WASHING EGGS UNDER CONDITIONS OF INCREASING OR DECREASING INTERNAL TEMPERATURES UPON HATCHABILITY
 (Pritsker, 1941)

Experiment	No. eggs set	Percentage of eggs hatched		
		Controls (clean eggs)	Eggs washed under	
			Increasing internal temperature	Decreasing internal temperature
1	100	84.6	80.8	75.4
2	150	65.9	66.3	57.2
3	150	71.2	70.6	59.5

TABLE 22

EFFECT OF DISINFECTION OF EGG SHELLS WITH 0.5 PER CENT FORMALIN
 UNDER CONDITIONS OF INCREASING AND DECREASING
 INTERNAL PRESSURE
 (Pritsker, 1941)

Experiment	No. eggs set	Percentage of eggs hatched		
		Controls (not dis- infected)	Disinfected under	
			Increasing internal egg pressure	Decreasing internal egg pressure
1	100	84.6	87.3	80.9
2	150	65.9	62.5	54.0
3	150	71.2	69.8	63.7

tions that increased internal pressure (table 22). An increasing internal pressure prevents penetration of the washing solution through the pores of the shell, whereas decreasing internal temperature and pressure tend to draw the solution into the egg.

THE ABSORPTION OF TOXIC SUBSTANCES

That the egg may absorb certain poisonous substances which will prove toxic to the embryos and result in failure to hatch was shown by Deakin and Robertson (1933), working with mercurial ointment. They found that eggs incubated under hens that had been treated for lice with mercurial ointment failed to hatch and that the embryos died before the third day of incubation. Deakin and Robertson then coated eggs with this ointment and incubated them artificially; only a few embryos survived this treatment. Fétré (1899) reviewed his own previous publications showing that turpentine, ammonia, mercury, phosphorus vapors, and alcohol had detrimental effects upon the hatchability of eggs exposed to them before incubation. Ether and chloroform had a temporary effect which disappeared if the eggs were allowed to rest after treatment and before incubation.

Landauer (1945) reported data which showed that the incidence of rumplessness was increased by the injection into eggs previous to incubation of insulin, cysteine hydrochloride, cystine, DL-glutamic acid hydrochloride, thioglycollic acid, and L-malic acid. Fétré (1899) listed a large number of materials which upon injection into eggs produced abnormal embryos.

THE IRRADIATION OF HATCHING EGGS

It was found by Bless and Romanoff (1943) that unincubated eggs irradiated with X rays of sufficient strength lose their hatching power (table 23). Moderate doses of X rays seemed to exert stimulating effects on the embryos, a dose of about 250 r. units being most effective.

Funk in 1931 conducted experiments (the results of which have not been published) on the problem of the effect of X rays on sex ratios in which X-ray dosages of 250 r. units, 500 r. units and so on, up to 2500 r. units were used. He found that when he

TABLE 23

X-RAY DOSAGE AND HATCHABILITY
(Bless and Romanoff, 1943)

X-ray dose (r. units)	Percentage hatchability of eggs
0	85.2
8	77.7
50	62.5
250	83.3
1000	40.0
2500	25.0
5000	0.0

exposed 75 eggs from mated pens to an X-ray dosage of 2500 r. units all eggs appeared infertile when incubated, as contrasted to 3 infertiles in 76 untreated eggs. There is no doubt that other types of irradiation would also devitalize fresh-laid fertile eggs.

AIR TRANSPORTATION AND ATMOSPHERIC PRESSURE

Through the development of modern air transportation the shipping of valuable hatching eggs by air has become a practical means of transport. Jull (1945) reported the results of two tests in which hatching eggs were shipped from Washington, D. C. to Los Angeles and return, and to Miami and return. The maximum altitude in these flights was 12,000 feet. Hatchability was not impaired by these shipments. Shipments to South American countries gave somewhat lower hatchabilities, the range being from approximately 50 to over 80 per cent.

Fraps (1945) investigated the effects of reduced atmospheric pressure on hatchability by simulating altitudes from 6500 feet to 90,000 feet. He did not get any significant reduction in hatchability until the pressure was reduced to 0.5 inches mercury (the equivalent of an altitude of 90,000 feet). Eggs held at this pressure for 12 hours daily for 3 days gave a hatchability of 45 per cent as compared to 92 per cent for eggs held at an altitude of 200 feet. The lower hatchability was associated with an excessive loss of water from the eggs.

Nelsen (1946) reported that increasing the atmospheric pressure during incubation to one and one-half times normal resulted in the death of the embryos, most of them dying between the sixth and tenth days and only 14 per cent surviving to the seventeenth day of incubation. He reported hatching chicks from eggs that were held under one and one-half atmospheres of pressure up to the fifth or sixth days of incubation; however, high mortality occurred among these chicks within 2 days after hatching. This work suggests the need for additional experiments to establish the effect on hatchability of increased atmospheric pressure during the holding period.

SUMMARY AND RECOMMENDATIONS

Our present knowledge of the care of hatching eggs justifies the following conclusions and recommendations:

1. Hatching eggs should be held where the temperature is 50° F. to 60° F. (Serious losses may result if hatching eggs are held at lower or higher temperatures.)
2. Eggs should be placed in incubators as soon as convenient after being laid and not longer than 7 to 10 days. (Hatchability declines rapidly when eggs are held longer than 1 week under average farm conditions.)
3. For best results hatching eggs should be held where the relative humidity is high (between 80 and 90 per cent), but below the point where mold develops on the eggs, cases, fillers, or flats.
4. Hatching eggs need not be turned while being held, unless they are held for periods longer than 1 week.
5. Eggs intended for hatching purposes should be handled carefully, being held in cases with the small ends of the eggs down. Best results are obtained with new cases, fillers, and flats and by the use of rigid pack.
6. Soiled hatching eggs may be dry cleaned or cleaned by washing in warm water or in disinfecting solutions held at temperatures above that of the eggs.
7. Hatching eggs should not come into contact with grease or oil, which tends to seal the shell, or with poisonous substances which may prove toxic to the embryo.

It is evident that the research work done in this field has been sketchy and for the most part very limited in the numbers of eggs used. In order to verify or disprove some of the results reported and to investigate aspects of this problem not previously investigated, it will be necessary to carry out experiments under well-controlled conditions with large numbers of eggs so that the significance of small differences may be established. Such experiments require more liberal support than has been available in this field in the past.

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CHAPTER 5

Biochemistry of the Developing Avian Egg

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that are to be transformed into the chick are present at the beginning of incubation. As incubation proceeds, it is possible to follow with considerable exactitude the disappearance of many materials from the egg, and their reappearance in the same or different form in the embryo and in the extra-embryonic membranes. The amounts of carbohydrates, proteins, lipids, and minerals present at various stages of development in the embryo and in the still unused portion of the egg indicate the approximate rate at which the embryo utilizes the nutriment provided by the egg. Additional information regarding embryonic metabolism can be obtained by measuring the rate of respiration and by determining the amount of heat that is produced. Lastly, a study of the functional activity of the extra-embryonic membranes discloses how mediation is accomplished between the embryo and its environment (including the environment within the egg) and also reveals the nature of catabolic processes during development.

It is fortunate for those interested in artificial incubation that much of the experimental work on the biochemistry of the embryo has been done on chicken eggs. Because of their abundance, chicken eggs were used in many classical studies of matter and energy metabolism. From these studies has come most of our present knowledge of avian development, under both normal and adverse conditions. However, many important questions are still unanswered. The following review will touch only upon the most fundamental discoveries.

SPECIFIC FEATURES OF AVIAN DEVELOPMENT

In the embryonic development of birds, there are a number of distinctive features. Because the avian embryo develops in isolation from the maternal environment, the structural adaptations found in the incubating egg are somewhat different from those associated with the embryos of viviparous animals. Furthermore, the type and the amount of food that the bird's embryo is to receive is not supplied by the mother from day to day but is determined in advance at the time the egg is formed.

The Role of Environment

The avian embryo exists in closer relationship to the external environment than the mammalian embryo. The normal em-

CHAPTER 5

Biochemistry of the Developing Avian Egg

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The Role of Environment

The avian embryo exists in closer relationship to the external environment than the mammalian embryo. The normal em-

bryonic development of the bird requires the proper environmental conditions. Temperature, relative humidity, and the supply of oxygen are especially important.

The avian embryo is dependent entirely upon the environment for the oxygen which it uses, except, perhaps, during the very early stages of development. Oxygen enters the egg through the shell, and carbon dioxide—the waste product of respiration—is

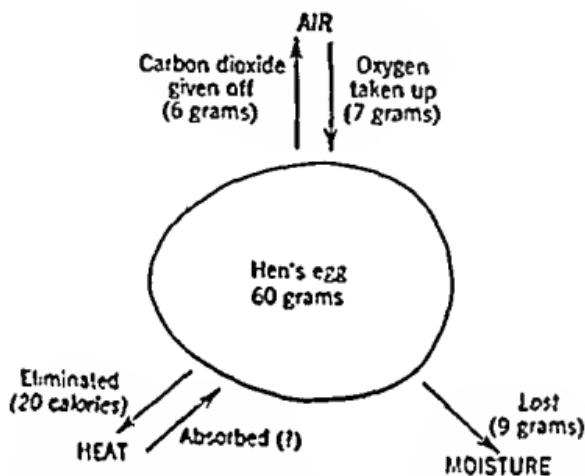


FIG. 45. Quantities of heat, moisture, and gases interchanged between the developing hen's egg and the environment during the entire period of incubation.

only a few degrees from the optimum. For example, the chicken embryo, if it is incubated at a temperature as low as 34.5° C. or as high as 39.5° C., grows at a retarded rate (Romanoff, Smith, and Sullivan, 1938), because the assimilation of the yolk is delayed and that of the albumen is never completed (Romanoff, 1943a). Although the rate of fat absorption is nearly normal at these temperatures, the final fat content of the embryo's body is somewhat low, as is, also, the calcium content (Romanoff and Faber, 1933). High and low incubating temperatures, respectively, advance the hatching date of chicks 1 day or retard it by as much as 4 days (Romanoff, Smith, and Sullivan, 1938). Similar acceleration and delay in hatching, due to variation in incubating temperature, have been observed with pheasants and quail (Romanoff, 1934), turkeys (Romanoff, 1935), and ducks (Romanoff, 1943c).

Extremes of humidity (for example, 40 per cent and 80 per cent relative humidity) result in embryonic growth at an irregular rate. When the humidity is too high, there is excessive absorption of moisture, and the growth rate is slightly accelerated, particularly during the latter part of the incubation period. The percentage of dry matter in the embryo's body is low, but the total ash is high, and calcium deposition is much favored. High humidity may cause a mortality rate of 60 to 80 per cent; low humidity, a mortality rate of 25 to 40 per cent (Romanoff, 1929; 1930b). Only about 30 per cent of duck eggs hatch under abnormal conditions of humidity (Romanoff, 1943c). The shell membranes, the allantoic sac, and the yolk sac are unusually dry or unusually watery, depending upon whether the humidity is too low or too high. As a result, the metabolism, normal physical activity, and respiration of the embryo may suffer interference. A high rate of air movement in the incubator may hasten evaporation of moisture from the egg contents. Air movement is particularly important at the time of hatching, when, if it is too rapid, it may quickly dry out the membranes of the egg. Excessive air movement may delay the hatching of ducklings several days (Romanoff, 1943c).

The effect of the gaseous composition of the air upon the metabolism of the embryo is shown by the changes in the growth rate that occur when the carbon dioxide content of the air is

increased. If the embryo is constantly exposed to a carbon dioxide concentration of 0.4 per cent, growth is stimulated in the early stages of incubation and retarded during the latter part of the developmental period. Higher concentrations of carbon dioxide adversely affect growth from the beginning (Romanoff, 1930c; Romanoff and Romanoff, 1933b). Ammonia is also very detrimental to the developing embryo (Féré, 1899).

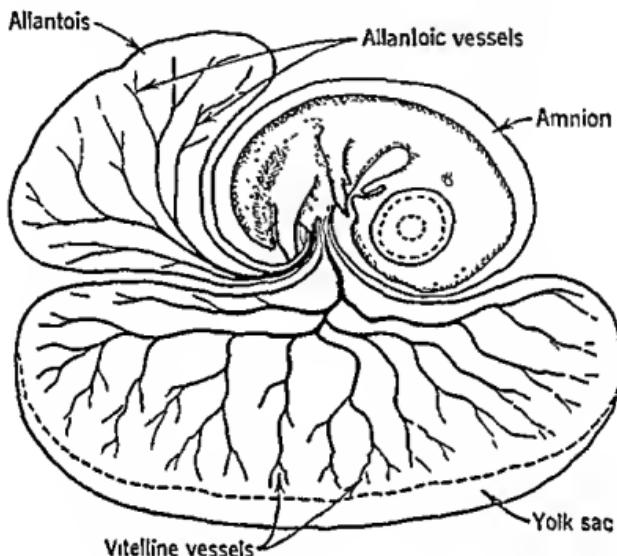


FIG. 46. The 12-day-old chick embryo and its extra-embryonic structures.
(After Duval, 1889.)

Structural Adaptation

The development of the avian embryo is accompanied by the appearance of special membranes which assist in embryonic metabolism. These structures provide for embryonic nutrition, respiration, and excretion until the time arrives when the chick can safely assume existence outside the egg.

The extra-embryonic structures appear early, but their formation is not completed until approximately the middle of the incubation period. Since their development is quite separate from that of the embryo proper, they are easily recognized in an opened egg (fig. 46). The biochemical functions of the most important membranes, the allantois, the amnion, and the yolk sac, are essential to the health and well-being of the embryo.

Food Supply

During the entire period of embryonic development, there is no food supply other than that already present in the egg. The most elaborate organization of all metabolic processes is necessary for the transformation of the raw materials of the egg into the living tissues and organs of the chick. The food supply is extremely complex and is chemically complete for the developing embryo.

At the beginning of development, there is only a minute cluster of living cells in the egg; these are situated in the blastoderm. The remainder of the egg—yolk, albumen, and shell—provides for the nutrition and protection of the embryo. Within a brief period of 21 days the mass of inert food material is converted into a chick. During the embryonic period the metabolism of any species is more efficient than at any other time of life. The exceedingly rapid rate of avian development is one indication of this fact.

GROWTH AND METABOLISM

The growth rate of the embryo corresponds approximately to the rate at which the contents of the egg disappear. The assimilation of the egg's individual components proceeds in an interesting and significant manner.

How the embryo grows at the expense of the egg's contents is shown better by the changes in dry weights of the embryo and of the egg components than by the changes in wet weights. Wet weights largely reflect the movement of water within the egg. Almost from the beginning of incubation, loss of water causes a sharp decrease in the weight of the albumen. Water diffuses from the albumen into the yolk; water is also absorbed by the embryo, used in the formation of the amniotic and allantoic fluids, and lost by evaporation to the exterior. The weight of the yolk is increased by the additional water from the albumen and does not start to decline until the end of the first week. These changes and the great increase in the weight of the embryo in the last week of incubation are shown in figure 47.

Dry-weight measurements, on the other hand, clearly indicate how the embryo draws upon the substance of the egg. They also

reveal that the absorption of the albumen does not parallel that of the yolk. The utilization of the yolk, gradual from the start, is greatly accelerated during the last 5 days of incubation. Between 25 and 35 per cent of the yolk remains unused at the time

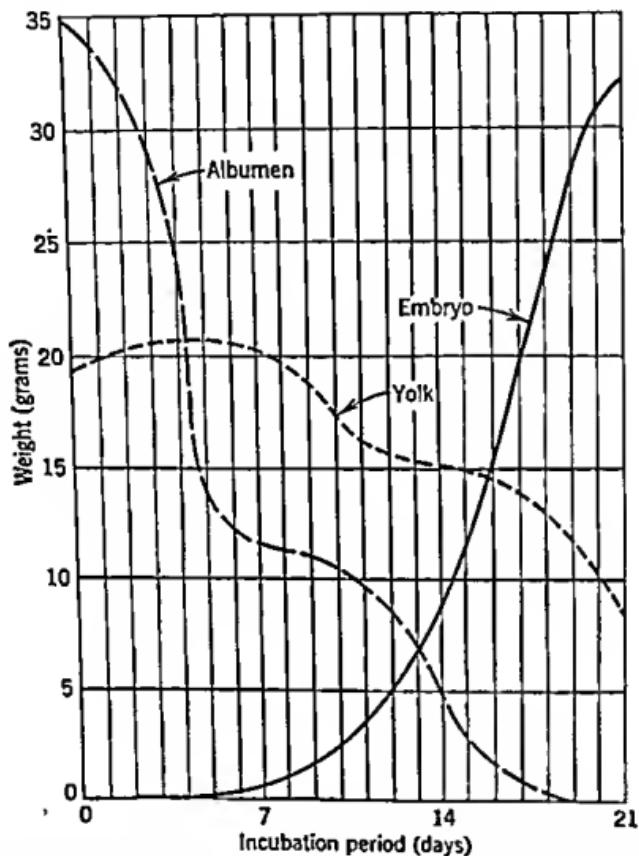


Fig. 47. Changes in the wet weight of the embryo, yolk, and albumen during the entire period of incubation of the chicken egg. (After Romanoff and Romanoff, 1933a.)

of hatching; this portion, with the yolk sac, is drawn into the body of the chick and is assimilated during the first week of postembryonic life. The dry matter of the albumen, unlike that of the yolk, remains almost undiminished during the first 9 days of incubation. After decreasing slightly in amount from the ninth to the twelfth day, the albumen is rapidly and completely absorbed. These changes in the dry weights of the egg's com-

ponents, and the almost reciprocal increase in the dry matter of the embryo, may be seen in figure 48.

During the first week of development, the embryo preferentially utilizes carbohydrates; during the second week, proteins; and during the third, fats. The absorption of proteins and fats coincides, respectively, with the utilization of the albumen, which

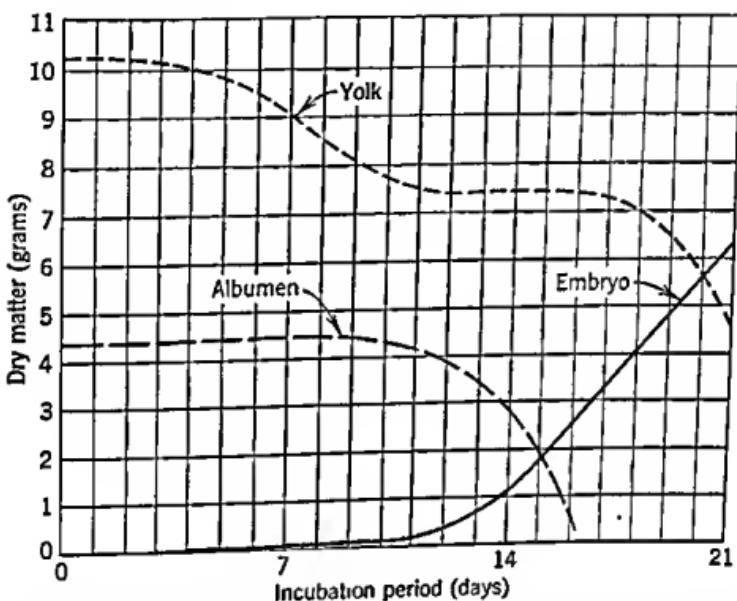


FIG. 48. Changes in the dry-matter content of the embryo, yolk, and albumen during the entire period of incubation of the chicken egg. (After Romanoff, Smith, and Sullivan, 1938; and Romanoff, 1943b.)

is largely protein, and of the yolk, which contains practically all the fats of the egg.

Carbohydrate Metabolism

The total carbohydrate of the chicken egg does not exceed 0.5 gram (Needham, 1927; Donhoffer, 1933). The importance of carbohydrates in avian embryonic development should not be overlooked, however, for they enter into the structure of new tissues and assist in the functional activities of the embryo.

In the egg, the carbohydrates exist both in the free state, as glucose, and in combination with proteins (glycoproteins) and lipids (phospholipids). About 75 per cent of the carbohydrates

are contained in the albumen, the remaining 25 per cent in the yolk.

In the developing chick, carbohydrates are found in the blood in the form of glucose and, after the seventh day, in the liver as glycogen. Quantitative estimations of the total carbohydrates in the incubating egg reveal that there is a regular increase in the amount of carbohydrates in the body of the embryo. In the remainder of the egg, however, the carbohydrate decreases until the seventh day, then sharply increases until approximately the tenth day (Donhoffer, 1933), and finally declines steadily until the end of the incubation period. In the yolk, lactic acid rises rapidly during the first week, then decreases to its initial value by the twelfth day; in the albumen, it increases during the first 3 days, but returns to the initial level by the fifth day (Capraro and Fornaroli, 1939).

The gain in total carbohydrate between the seventh and the eleventh days coincides with a loss of fat. There is probably an interconversion of sugar and fat during early incubation (Capraro, 1941).

Protein Metabolism

In the contents of the fresh chicken egg, there are about 6.6 grams of protein, of which approximately 3.5 grams are in the yolk and 3.1 grams in the albumen. In the yolk, there are two proteins; in the albumen, at least five. According to Ono (1936), the two yolk proteins are absorbed at an equal rate, but the ovomucoid is utilized more rapidly than the other proteins of the albumen. The proteins of the egg serve as the source of amino acids for a new set of proteins, those of the embryo's body. Large portions of the egg proteins are utilized for the formation of embryonic tissues; other portions serve to provide energy, and eventually disappear (Schenck, 1932). About 90 per cent of the crude protein of the egg is recoverable from the body of the hatched chick (Gru, 1917).

The changes in the amounts of a number of amino acids have been followed in the yolk, the albumen, and the embryo. Among these amino acids are tryptophane, tyrosine, phenylalanine, arginine, lysine, and cysteine. In general, all these amino acids diminish in the yolk and the albumen (but to a greater extent

in the latter), and increase in the embryo, especially during the last 5 days of incubation (Sendju, 1925, 1927a). Tryptophane decreases in the extra-embryonic part of the egg during incubation, then increases, and later decreases again. In the opinion of Sendju (1925), the two sudden diminutions are perhaps coincidental with the formation first of hemoglobin and then of bile pigment. There is some evidence that arginine may be converted into histidine (Kamachi, 1935) and creatine (Palladin and Rashba, 1937). Creatine disappears from the albumen and yolk after the fourteenth day of incubation, and increases nearly a hundredfold in the embryo between the seventh and the nineteenth days (Sendju, 1927b). Cystine practically disappears from the yolk by the nineteenth day, when most of the cystine of the egg has been transferred to the embryo (Sendju, 1927a). Glycine is possibly synthesized during development (Patton, 1937). Sendju (1925) found that the total amounts of tryptophane, tyrosine, and arginine had decreased at the end of incubation, whereas slightly more than the original amount of histidine was present, and the quantity of lysine remained unchanged. The newly hatched chick contains about 65 per cent as much phenylalanine and tyrosine as the unincubated egg (Grau, 1947).

As the embryo grows, some proteins are used for energy, and their decomposition products accumulate in the allantoic sac. These products are chiefly ammonia, urea, and uric acid, in order of appearance and of increasing amount (Needham, 1926a, 1926b, 1926c). They are formed at maximum rates on the fourth, ninth, and eleventh days, respectively. The peak in protein catabolism occurs at about 8.5 days, between the periods of maximum utilization of carbohydrate and fat. The total nitrogen of the egg at the end of incubation is about the same as at the beginning, but the nonprotein nitrogen has increased at the expense of the protein nitrogen, because of the combustion of some of the proteins (Iljin, 1917; Idzumi, 1924).

Lipid Metabolism

Egg yolk is the chief source of lipids for the developing embryo. During incubation, the ether-soluble fatty substances in the chicken egg diminish from 5.8 grams to 1.9 grams (Romanoff,

1932); in other words, about 3.9 grams of fat are utilized in the process of embryonic development.

Some time ago, Tangl and von Mituch (1908) came to the conclusion that only about 28 per cent of the egg's original lipid content was deposited in the tissues of the embryo. They found that 40.5 per cent was burned—that is, used chiefly for the production of heat—and that about 31.5 per cent remained in the yolk at the end of the incubation period. The amount of fat burned, as they determined it experimentally, is in good agreement with the amount calculated by Murray (1925) from the carbon dioxide output, if it is assumed that the latter is derived entirely from the oxidation of fat.

Presumably very little fat is used by the embryo up to approximately the twelfth day. After this time, the fat of the egg is rapidly utilized, and the amount of fat increases in the body of the embryo, especially during the week before hatching.

It has also been observed (Riddle, 1916) that the phospholipids are utilized more rapidly than the neutral fats. In the embryo, the amount of phospholipid rises continuously, whereas the amount of neutral fat increases only after the fourteenth day of incubation. The daily increments of total phospholipid and cholesterol are greatest at about the fourteenth day and the seventeenth day, respectively (Cahn and Bonot, 1928). The most important phospholipids of the egg, lecithin and cephalin, increase at parallel rates; the initial lecithin-cephalin ratio of 3 to 1 is maintained throughout the developmental period (Kugler, 1936).

It has been shown (Hevesy, Levi, and Rebbe, 1938) that, after radioactive phosphorus is introduced into the incubating egg, the phospholipid in the embryo becomes very radioactive whereas that in the yolk remains inactive. These findings indicate that the embryo synthesizes its phospholipids, instead of absorbing them without change.

Quantitative differences in the fat content of the bird's diet have no significant effect on the hatchability of eggs (Heywang, 1942). It is possible, however, that variation in the quantity and composition of the egg fats may influence the composition of the body tissues and perhaps the health of the chick.

Mineral Metabolism

An adequate complement of minerals is necessary in the egg if the chick is to hatch. In the contents of the unincubated chicken egg there is normally about 0.4 gram of mineral material, almost equally divided between the yolk and the albumen. The yolk, however, contains more phosphorus, magnesium, calcium, and iron than the albumen, which is richer in sulfur, potassium, sodium, and chlorine than the yolk. In the egg shell there are approximately 5.9 grams of minerals, of which more than 98 per cent consists of salts of calcium.

During the first week of incubation, the inorganic salts of the albumen diffuse into the yolk; but from the seventh day on, the ash content of both the albumen and the yolk decreases steadily as that of the embryo increases. At various times, minerals are present in the embryo's body in different proportions. Potassium, sodium, and chlorides predominate during the early stages of development; calcium, phosphates, and sulfates during the later. Eventually, 75 per cent to 95 per cent of the major minerals of the egg contents are utilized by the embryo. It is interesting to note that as the absolute amount of minerals in the embryo increases, the relative amount (in percentage of dry weight) declines, until the sixteenth or seventeenth day (Romanoff, 1930a).

The egg shell provides the largest part of the calcium which is deposited in the body of the embryo. The newly hatched chick (exclusive of the yolk sac) contains about 150 milligrams more calcium than the entire contents of the unincubated egg. The transfer of calcium, as bicarbonate, from the shell to the embryo is made possible because carbon dioxide and water are given off during incubation. Between the tenth and the fifteenth days of incubation, when heavy ossification of the embryonic skeleton begins, the calcium content of the embryo increases sharply. According to Kroon (1940), the soft tissues nevertheless gain calcium at a relatively more rapid rate than the skeleton during the last week.

Calcium metabolism is intimately related to phosphorus metabolism. Like calcium, inorganic phosphorus markedly increases at about the tenth day (Baldwin and Needham, 1933). The calcium-phosphorus ratio in the embryo's body is less than

1 until late in the incubation period, at which time it exceeds unity (Insko and Lyons, 1933). As Plimmer and Scott (1909) pointed out many years ago, the transfer of phosphorus from the egg to the embryo necessitates a transformation of the egg's phosphorus compounds. The phosphorus of the egg contents is almost all organic, and largely ether-soluble, whereas more than half of the phosphorus of the embryo is inorganic. Hevesy, Levi, and Rebbe (1938) have shown that inorganic phosphorus radicals are split off from organic compounds and utilized. During the last week of incubation, the formation of lipoid and nucleoproteid phosphorus, as well as of inorganic phosphorus, increases in the soft tissues. Phosphatase is apparently necessary for phosphorus metabolism not only in the bones but also in the soft tissues (Sloot and Kroon, 1942).

During development, iron and copper diminish in the blood of the embryo, copper the more rapidly (Kamegai, 1939); after the tenth day, both increase in the liver. More than half of the iron in the liver is in nonhematin form (McFarlane and Milne, 1934). About 96 per cent of the egg's total iron is transferred to the embryo (Szejnman-Rozenberg, 1933). At the time of hatching, nearly all the copper of the embryo is concentrated in the liver (Loeselike, 1931).

Potassium is assimilated by the embryo at a fairly regular rate. Ninety per cent of the amount originally present in the egg is absorbed (Leulier and Paulant, 1935).

The accumulation of manganese is most rapid from the ninth to the fifteenth day and has almost ceased by the eighteenth day. About 75 per cent of the manganese of the yolk is utilized (Gallup and Norris, 1939).

Changes in Vitamin Content

Experimental findings have clearly indicated that the development of the chick embryo is not carried to completion in the absence of a normal complement of certain vitamins in the egg, notably riboflavin and vitamin D. Other vitamins are also important, a fact which becomes evident when the course of their transfer from the egg contents to the embryo is followed.

During embryonic development, the metabolism of the various vitamins differs. Of the water-soluble vitamins, thiamin and

pantothenic acid—which appear to be involved in hemoglobin formation (Taylor, Mitchell, and Pollack, 1941)—remain unchanged in amount at the end of incubation, as does riboflavin. One of the effects of riboflavin deficiency is myelin-sheath degeneration (Engel, Phillips, and Halpin, 1940). The quantity of biotin, also, is the same at the beginning and at the end of incubation (Snell and Quarles, 1941). On the other hand, the embryo possesses the ability to synthesize inositol, the concentration of which rises at least 500 per cent (Snell and Quarles, 1941). Nicotinic acid, too, is synthesized; after the eleventh day of incubation, it gradually increases in amount until ten or twenty times the original quantity is present in the egg (Dann and Handler, 1941; Snell and Quarles, 1941). Ascorbic acid (vitamin C), almost entirely absent from the fresh egg, may be detected in the extra-embryonic tissues between the first and the third days of incubation (Barnett and Bourne, 1941). It appears in the embryo on the fourth day (Ray, 1934), increases during the next 10 days, and, after a temporary decrease, eventually reaches its maximum in the liver of the newly hatched chick (Suomalainen, 1939a).

The quantitative changes in the fat-soluble vitamins has received little attention. Suomalainen (1939b) estimated that the two-week-old embryo contained 6.3 per cent of the total vitamin A of the egg, and that the vitamin A content of the yolk decreased by 45 per cent in the second and the third weeks of incubation.

Activity of Enzymes

Enzymes are as important in embryonic development as they are in the metabolism of adult animals. In the incubating hen's egg, a large variety of enzymes has already been found, and there are doubtless many more which still remain to be detected. They are distributed among the yolk, the albumen, and the embryo proper and take part in the breakdown of the egg materials and in the synthesis of embryonic tissues, as well as in many of the life processes of the developing chick.

Among the enzymes found by early workers both in the yolk and in the albumen are lipase, protease, catalase, lecithinase, carotenase, ovomucoidase, and amylase. Koga (1923) identified histozyme (hippuricase), salicylase, and tyrosinase in the yolk.

alone. The presence of an antitryptic enzyme in the albumen has been well known since its discovery by Sugimoto in 1913. During incubation, most of these enzymes increase in activity, especially the lipase, protease, and catalase of the yolk (Tallarico, 1908), but the antitrypsin of the albumen gradually disappears (Idzumi, 1924). The protease and lipase are at their maximum activity at the time of maximum protein and fat catabolism, respectively (Remohti, 1927, 1930).

The embryo, at the beginning of development, is supplied with at least three fundamental enzymes, a lipase, an amylase, and a protease (Galvialo, 1926), as well as with an alkaline and an acid phosphatase (Moog, 1944). Dipeptidase (Palmer and Levy, 1940) and aminopeptidase (Levy and Palmer, 1943) are demonstrable in the embryo at 72 hours. Later, catalase, peroxidase, urease, and pepsin make their appearance. The proteolytic enzyme, cathepsin, is demonstrable in the embryonic membranes on the ninth day, and quickly attains its highest value (Goldstein and Gintzburg, 1936). Carbonic anhydrase has been detected in the eye vesicle on the fourth day, and in the blood on the eleventh day, coincidental with an increase in carbon dioxide output (van Goor, 1940). Adenylpyrophosphatase increases from the sixth day to the twelfth day (Moog and Steinbach, 1945). Alkaline phosphatase, however, rises from the second to the fourth day, declines up to the sixth day, and reaches a maximum at 10 days. It is concerned in bone deposition and is present in greater concentration than acid phosphatase. The latter is at the maximum at 6 days, and then declines (Moog, 1946). The decrease in phosphatase activity appears to be correlated with the progressive differentiation of embryonic tissues (Moog, 1944). Cytochrome oxidase activity rapidly increases after the eighth day, as fat utilization rises (Albaum and Worley, 1942) but may be detected as early as the first day (Moog, 1943). Succinoxidase appears somewhat later than cytochrome oxidase (Albaum, Novikoff, and Ogur, 1946). Catalase activity increases in inverse relationship with anaerobic glycolysis (Kleinzeller and Werner, 1939). Auxins have been found to increase from 57 plant units to 40,000 plant units in 14 days (Robinson and Woodside, 1937). The diastatic and lipolytic activity of the embryo's

liver and the proteolytic activity of its stomach increase as development proceeds (Galvialo and Goryukhina, 1937).

The Role of Hormones

Hormones are of undoubted importance in embryonic development; in fact, their activity is probably fundamental and directly related to the gene mechanism. However, this aspect of hormonal influence needs clarification. Of the better known hormones it is probable that only two, estrin (Marlow and Richert, 1940) and insulin or an insulin-like substance (Shikinami, 1928), are present in the unincubated egg, although Tsuji (1922) detected a thyroid-stimulating principle also. During the first few days of incubation the estrin content of the egg decreases, and at the time of hatching estrin is found in the chick of either sex in the amount of about 0.004 milligram (Riboulleau, 1940-1941). The insulin of the egg quite possibly takes part in the active carbohydrate metabolism of the early developmental period, before the embryo is capable of secreting its own insulin. Adrenalin makes its appearance in the embryo at about the ninth day. The embryonic pituitary becomes active during the latter half of incubation; it secretes growth, thyrotropic, and gonadotropic hormones, which influence the body size of the chick, the development of the thyroid, the plumage, and the gonads (Fugo, 1940).

Pigment Metabolism

One of the most remarkable synthetic processes in the developing egg is the elaboration of pigments. The fresh egg contains only a few pigments, otoporphyrin in the shell and shell membranes, carotenoid pigments in the yolk, and ovofavin in the yolk and the albumen. During embryonic development, the specific pigments of the blood, the bile, and (in some breeds or varieties) the down and the skin are formed in abundance. These pigments bear little resemblance to the pigments of the fresh egg.

The synthesis of hemoglobin, the red porphyrin pigment of the blood, is very striking, although little is known concerning the actual mode of synthesis. Porphyrin increases in the first week of incubation from less than 4 micrograms to nearly 140 micrograms and may be found in the yolk and the albumen as well as in the embryo itself (Hijmans van den Bergh and Grotewiel,

pass, 1936). It is not derived from the shell. Coproporphyrin I, a related pigment, is synthesized much more slowly than hemoglobin. At the end of incubation, there is about 20,000 times as much hemoglobin as coproporphyrin I; the two seem to be synthesized independently (Sehønheyder, 1938).

The bile pigments similarly increase during development, but they do not appear in the chick before the seventh day of incubation (Sendju, 1927c). They are derived from the breakdown of hemoglobin.

Studies on the origin of the carotenoid pigments of the chick's retina (Wald and Zussman, 1938) indicate that three pigments are formed during the third week of incubation. These pigments are a red one containing astacin, a golden yellow one containing xanthophyll, and a greenish yellow one containing a hydrocarbon. With the exception of xanthophyll, these are synthesized by the embryo. Carotenoid pigments (as distinct from the carotenes) do not appear to be essential to normal embryonic development. Palmer and Kempster (1919) hatched healthy chicks from eggs entirely devoid of these pigments; the only abnormality in the chicks was the complete absence of yellow pigmentation in the skin.

Very little is known about the chemical metabolism of the melanin pigments responsible for the color of the down. The synthesis of melanins is undoubtedly under genetic control and apparently is dependent upon enzymatic activity also. A few morphological studies have been made of the origin of melanins in the chick (Dorris, 1938; 1939).

RESPIRATION AND HEAT PRODUCTION

The synthetic processes of embryonic development require the expenditure of energy, which is provided by the combustion of the egg's food materials in oxygen derived from the atmosphere. The production of heat and the elimination of carbon dioxide by the incubating egg give evidence that the potential energy of the raw egg materials has been transformed into the kinetic energy of the embryonic tissues. The metabolic rate of the embryo, as measured either by the amount of heat given off or by the amounts of oxygen consumed and of carbon dioxide evolved, is

correlated with the embryo's age and weight and therefore changes continually throughout the incubation period.

Gaseous Exchange

Embryonic respiration is nonpulmonary until the seventeenth day. During the earliest stages of incubation, it is possible that

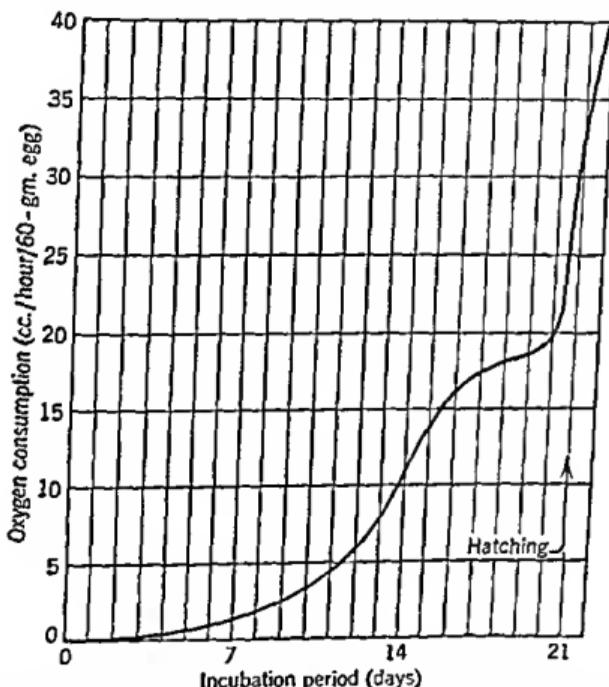


FIG. 49. Changes in the hourly amount of oxygen used by the chick throughout the period of embryonic development and on the first day after hatching. (After Romanoff, 1941.)

the embryo utilizes oxygen derived from the yolk through the activity of the enzyme catalase (Needham, 1931, page 700). Later, the allantoic vessels assume their respiratory function, and the oxygen which is used is of atmospheric origin. The hourly consumption of oxygen by the embryo increases from the beginning; it is 0.51 cubic centimeter at the start and 17.34 cubic centimeters on the seventeenth day, after which time it levels off, as shown in figure 49. When the chick hatches, its utilization of oxygen increases enormously. The plotted curve of oxygen ab-

sorption follows closely the curve of embryonic growth. However, the metabolic rate of the embryo, expressed in terms of the volume of oxygen consumed per hour per gram of body weight, decreases from about 48 cubic centimeters on the second day to 1.5 cubic centimeters on the eighth day. Thereafter, it declines to a minimum of 0.64 cubic centimeter on the day before hatching (Romanoff, 1941).

When the egg is laid, it contains dissolved carbon dioxide, which immediately begins to be dissipated into the external atmosphere. The carbon dioxide output of the embryo is therefore obscured, for a day or two, by the escape of the egg's carbon dioxide. Subsequently, the hourly elimination of carbon dioxide by the embryo increases throughout incubation, paralleling the consumption of oxygen (Bohr and Hasselbalch, 1900). On the third day, 0.074 cubic centimeter of carbon dioxide is produced; on the seventeenth, 10.8 cubic centimeters. The amount of carbon dioxide evolved per hour per unit weight of embryo, like the amount of oxygen absorbed, declines sharply until approximately the eighth day. There is probably a slight increase between the ninth and the fourteenth days, followed by another decrease (Noyons and de Hesselle, 1939). Altogether, about 3 liters of carbon dioxide per egg are produced during the developmental period.

The respiratory quotient, or the ratio of the volume of carbon dioxide produced to the volume of oxygen consumed, changes in a significant fashion during incubation. The respiratory quotient provides an index of the character of the food substance which is being oxidized by an organism. Depending upon whether carbohydrates, proteins, or fats are oxidized, the respiratory quotient is 1.0, 0.78, or 0.7, respectively. The respiratory quotient of the chick embryo exceeds 0.8 only during the first week, when carbohydrate metabolism predominates. During the second week, when proteins are used abundantly, the respiratory quotient is much closer to 0.8 than during the third week, when it is chiefly fats that are utilized, the quotient being then in the neighborhood of 0.7.

Heat Production

Heat production during incubation keeps pace with oxygen consumption and carbon dioxide elimination. Bohr and Hasselbalch

(1903) estimated that 0.39 gram-calory per day was given off on the fourth day and 90 gram-calories on the nineteenth. During the entire developmental period, a total of 12 to 20 kilo-calories of heat are produced. The metabolic rate, in terms of gram-calories of heat given off per hour per gram of embryo, decreases rapidly until the ninth day; it then increases somewhat during the next 3 days, and later declines gradually.

At the end of the incubation period, only 15 to 20 per cent of the solid matter of the egg has undergone combustion.

PHYSIOLOGY AND CHEMISTRY OF EMBRYONIC MEMBRANES

All three extra-embryonic structures, the amnion, the allantois, and the yolk sac, are important to the development of the embryo. Each has its own specific physiological functions. During incubation, the contents of each change considerably in volume, specific gravity, hydrogen-ion concentration, and chemical composition.¹

The Yolk Sac

The yolk sac is nutritional in function. Its walls, surrounding the yolk (although not completely until the seventeenth day), absorb food materials into the vitelline circulation, through which the nutrients are carried to the embryo. On the last day of incubation, the yolk sac, with its greatly diminished contents, is drawn into the body cavity of the embryo.

The changes in the yolk throughout incubation have already been indicated. However, it may be added here that the yolk, originally slightly acid, achieves neutrality within a week and is distinctly alkaline at the end of the second week. During the last week, it grows less alkaline, and it is again neutral at the end of incubation (Romanoff and Romanoff, 1933a). (By contrast, the pH value of the albumen decreases from a high point on the second or the third day to its original value of 7.5 on the sixth day. Thereafter, there is a gradual approach to neutrality.)

The Amnion

The amnion, which appears slightly before the allantois, very soon becomes filled with fluid. The embryo is bathed in this

liquid and is thus cushioned against mechanical injury. The amniotic fluid also serves to protect the embryo from dehydration and from adhesion to the extra-embryonic membranes and to the shell membranes. In addition, part of the fluid is apparently swallowed by the embryo, during the late stages of development.

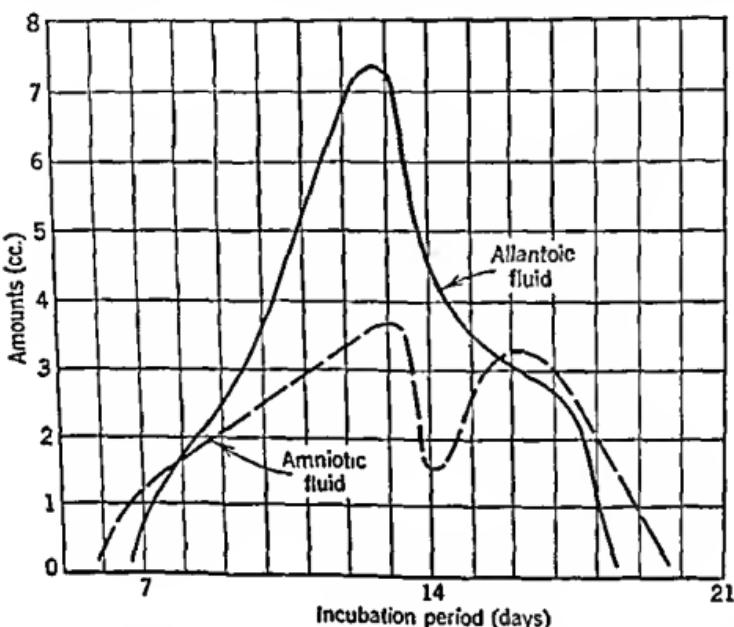


FIG. 50. Changes in the volume of the allantoic and amniotic fluids in the incubating chicken egg. (After Romanoff and Hayward, 1943.)

The amniotic fluid reaches its maximum volume (about 3 cubic centimeters) on the twelfth or the thirteenth day of incubation and thereafter diminishes gradually (fig. 50). Its specific gravity is highest (close to 1.06) a day or two after its quantity has reached the peak. Throughout the incubation period, it grows less alkaline steadily but slowly. At the beginning, its hydrogen-ion concentration corresponds to a $p\text{H}$ value in the neighborhood of 8; near the end, the $p\text{H}$ is less than 7.0 (Romanoff and Hayward, 1943). In the last 4 days, however, the fluid again becomes alkaline (Walker, 1943). Its electrical conductivity increases rapidly during the first 10 days, and thereafter remains fairly constant (Romanoff and Grover, 1936).

Kamei (1927) analyzed the amniotic fluid on the ninth day,

and again on the fourteenth day. The general composition of the fluid at these times was as given in table 24. Sodium and chlorine are the most abundant inorganic constituents, and both diminish by one-half in the interval between the ninth and the fourteenth days. The great increase in organic material in this period is caused by the mingling of the proteinaceous albumen with the amniotic fluid on the twelfth day, when communication is established between the albumen sac and the amnion at the

TABLE 24

COMPOSITION OF THE AMNIOTIC FLUID OF THE CHICK EMBRYO

	Day of incubation	
	Ninth day (gm./100 cc.)	Fourteenth day (gm./100 cc.)
Total solids	0.964	29.46
Organic material	0.034	28.57
Total ash	0.931	0.885

sero-amniotic juncture (Hirota, 1894). The influx of albumen also results in a slight increase in acid-soluble phosphates (Kugler, 1945). An absorption of proteins in the final stages of development, leaving predominantly basic constituents, may possibly account for the rise in *pH* in the last 2 days (Walker, 1943).

The Allantois

The allantois functions as a respiratory organ. Its blood vessels transport oxygen to the embryo and carry away the carbon dioxide which is eliminated. Into the fluid-filled allantois sac, also, are excreted the waste products of embryonic metabolism, especially those of the kidney.

The allantois fluid makes its appearance at about the fifth day, attains a maximum volume of 6 cubic centimeters (Fiske and Boyden, 1926) or 7 cubic centimeters (Romanoff and Hayward, 1943) on the fourteenth day, and thereafter decreases at a rate similar to its rate of increase (cf. fig. 50). Throughout the incubation period, but particularly during the last week, its specific gravity rises and eventually reaches a value of about 1.020. Accordinging to Kamei (1927), its total solids increase 4.5 times be-

tween the first and the second week, and the organic material increases nearly nine times. The fluid grows more acid as development proceeds, so that at the time of hatching the pH has declined from its original value of more than 8 to a final value of less than 6 (Romanoff and Hayward, 1943). The increase in acidity is accelerated during the last week (Walker, 1943). The electrical conductivity of the fluid, which rises rapidly during the first half of incubation, decreases sharply thereafter (Romanoff and Grover, 1936).

The continuous increase in the acidity of the allantoic fluid is a result of the respiratory and excretory function of the allantois. The accumulation of carbon dioxide is in part responsible for the change in pH. In addition, the enormously increased excretion of free uric acid during the last week decreases the free basic constituents of the fluid (Walker, 1943). The excretion of uric acid or its salts into the allantoic fluid is also the principal reason for the rise in the content of organic matter. As the fluid grows more acid, the uric acid is no longer soluble and is deposited on the walls of the sac. Other waste products, such as ammonia, urea, and creatinine, also increase during incubation, but to a lesser extent than uric acid.

POSTEMBRYONIC ABSORPTION OF THE YOLK SAC

When the chick is hatched it contains within its body that portion of the yolk not utilized during incubation. Depending upon the original size of the egg, the amount of unabsorbed yolk varies; in Leghorns, there are usually 5 or 6 grams of spare yolk (Entenmann, Lorenz, and Chaikoff, 1940; Romanoff, 1944). This remnant of the yolk continues to be assimilated and eventually disappears. Usually the greatest part of it has been used by the fifth or the sixth day after hatching. The yolk sac is a diverticulum of the small intestine; its contents are absorbed directly from the sac into the blood stream.

The dry matter of the yolk decreases in amount more rapidly than the total wet weight; this fact indicates that the actual food material is utilized (Romanoff, 1944). Both proteins and fats are speedily absorbed. During the first 36 hours after hatching, the yolk loses about 160 milligrams of nitrogen (Romenski, 1949).

The fatty acids, of which there is 0.5 gram at hatching, are reduced to less than 0.03 gram in 5 days. The quantities of phospholipid and total cholesterol—0.07 gram and 0.06 gram, respectively—decrease in a less striking manner; the removal of cholesterol esters lags considerably (Entenman, Lorenz, and Chaikoff, 1940).

The liver and the blood of the newly hatched chick contain large amounts of lipids, doubtless because the embryo derives so much of its nourishment from the yolk, which is rich in lipids. On about the third day after hatching the lipid contents of the blood and the liver start to decrease. The disappearance of excessive amounts of fat occurs at roughly the same time as the disappearance of the yolk sac (Entenman, Lorenz, and Chaikoff, 1940).

CONCLUSION

The brief review given above indicates that biochemists have investigated numerous phases of embryonic development. They have traced various organic and inorganic compounds throughout the developmental period, have noted the appearance of some and the disappearance of others, and have observed the processes that convert certain substances into entirely different ones.

Perhaps to the practical man, much of the investigation into the biochemistry of the developing egg appears haphazard and may seem to contribute little toward the production of better chicks or the improvement of hatchability. It must be admitted that there has been a lack of coordinated effort directed toward the solution of practical problems; but it must also be admitted that any real addition to our knowledge of avian development cannot fail to find eventual application.

By implication, biochemical research indicates that a good, hatchable egg contains a full complement of certain essential ingredients and that the proper environmental conditions release the latent energy in such an egg and initiate the transformations that result in a chick. Biochemistry thus serves two practical functions. It can reveal how the course of embryonic development is affected, on the one hand, by variation in the composition of the egg, and, on the other, by environmental conditions during incubation.

Investigation has already produced many fruitful results. There remain, however, an enormous number of possibilities for future exploration. For example, there is no doubt that many of the losses still sustained in hatching are correlated with the quality of the eggs obtained under the conditions of modern poultry management, which necessitate close confinement and more or less unnatural feeding of the flock. In addition, the well-being of the newly hatched bird obviously depends not only upon the composition of the egg but also upon the efficiency with which the contents of the egg were utilized during the bird's embryonic life. Since the chemical metabolism of the embryo is vitally affected by various physical factors, there is a great need for a more precise chemical knowledge concerning the influence of environment in the artificial incubation of chickens and other domestic birds. Storage conditions for hatching eggs also need investigation. In fact, biochemistry provides rich opportunities for productive work that should be of great value not only to the hatcheryman but also to the manufacturer of incubators.

Chemical research in avian embryology is a very broad field, but it is well adapted to the study of specific problems. The answers to many problems, gradually accumulated, may serve as steppingstones in the future progress of artificial incubation.

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CHAPTER 6

Physical Conditions in Incubation

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The study of physical factors in incubation is concerned primarily with temperature, humidity, the relation between temperature and humidity, turning, the position of the eggs, ventilation, and fumigation.

The determination of the optimum physical conditions for the incubation of chicken and turkey eggs is based upon a knowledge of embryonic development and the response of embryos to different test conditions. The hatcheryman usually measures the success of the incubation procedure employed by the hatchability of the eggs set. The research worker, on the other hand, is particularly interested in the effect of the conditions upon the embryo and upon the time and total amount of embryonic mortality. Mortality tends to be concentrated in certain days of the incubation period which are known as the peak periods of embryonic mortality (see chapter 7 for a discussion of mortality peaks). Throughout this chapter, recommendations for the use of certain physical conditions in incubation will be based on their influence upon the peaks of mortality as well as upon the total amount of mortality.

TEMPERATURE

Temperature is the most critical factor in determining success or failure in hatchery operation. Slight variations in temperature may mean the difference between high and low hatchability. In addition to temperature, the relationship between temperature and humidity must also be considered. Temperature has an influence not only on hatchability but also on time of hatching, embryo size, embryo mortality, size and viability of chicks and pouls, and percentage of crippled and weak chicks and pouls produced.

Optimum Temperatures

Particular care should be taken to operate the incubator at the exact temperature recommended by the manufacturer for that particular make and model.

In forced-draft incubators the temperature varies from 99° to 100° F., according to the incubator. When the hatching compartment is combined with the incubating compartment the same temperature is maintained throughout the 21 days. Where a separate hatcher is used the temperature may be reduced considerably in the hatcher. Temperatures recommended usually range from 96° to 98° F., and again it should be stressed that the manufacturer's directions should be followed closely.

In the natural-draft, sectional type of incubator or in the small single-sectioned, natural-draft machine an increased temperature each week is standard procedure. With the bulb of the thermometer on a level with the top of the eggs the temperature may read 101° F. for the first week, 102° F. for the second week, and 103° F. for the third week. Care should be taken that the temperature does not exceed 103° F. during the third week of incubation.

For turkey eggs, the same directions should be followed. In the natural-draft, sectional type of machine, the temperature should be 100.5° F. for the first week, 101.5° F. for the second week, 102.5° F. for the third week, and 103° F. for the fourth week. Again it should be remembered that a temperature higher than 103° F. for the last week will increase embryo mortality.

Experimental results on temperature requirements are quite consistent. In forced-draft incubation, Romanoff (1936) showed that when eggs were continuously exposed to a constant temperature results were best at 100.4° F., and good hatchies were secured at 99.5° F. and 98.6° F. Barott (1937) reported that best hatches were obtained with a temperature of 100° F. and that hatchability decreased at both higher and lower temperatures, at first slowly and then more rapidly, as the deviation from 100° F. increased. At 96° F. and 103.5° F., nearly all the embryos died in the shell. Later work by Romanoff, Smith, and Sullivan (1938) showed that best hatches resulted at a continuous temperature of 99.5° F. It was also found that lowering the temperature after the sixteenth day produced no ill effects, even when the temperature was lowered as much as 5.4° F. In general, the results indicate that the probability of a good hatch may be increased by slightly raising the temperature during the first part of incubation and considerably lowering it during the latter part.

— — — — —
at the upper limit

conditions for the different stages of embryonic development. It also suggests that there is an optimum environment for the development of the embryo inside the shell and for the conditions that influence the chick to break out of the shell." High hatchability would certainly be more prevalent if every incubator operator kept these facts in mind.

High Temperature Effects

A temperature above normal or optimum causes high embryonic mortality, especially during the last week of incubation. This mortality usually occurs on the nineteenth day for chicken eggs and on the twenty-fifth day for turkey eggs. The extent of mortality is dependent on the length of time that the high temperature is maintained and on the degree of the increase in the temperature above normal.

The action of the thermostat controlling the temperature in incubators should be checked at frequent intervals with a clinical thermometer of known accuracy, and all controls should be checked by a factory representative or returned to the factory for checking every 2 or 3 years. The actual temperature should be checked at the time the pilot light goes off, that is, when the thermostatic controls cut off the heat. The temperature should not be read when the heat is on, since the temperature may rise considerably higher thereafter and thus may reach the critical stage without the operator's knowledge. The temperature of a separate hatcher should be watched for a short time after the pilot light goes off in order to make certain that the temperature does not increase because of the heat generated by the eggs themselves.

There is ample evidence to support the contention that temperature is the most important physical condition in incubation and that temperatures above optimum are likely to be disastrous. As already mentioned, Barott (1937) found that the hatch decreased at higher temperatures and that at 103.5° F. in a forced-draft incubator nearly all embryos died in the shell.

One of the most significant experiments was that of Romanoff (1939) in which groups of embryos at various stages of incubation were exposed for 24 hours to temperatures of 105.8° F. or 84.2° F. in a forced-draft incubator. These experiments indicated that

the temperature effect was greatest in the early stages, particularly to the tenth day. The growth of the embryos was accelerated by high temperature and retarded by low temperature. The temperature effects were lessened as incubation progressed, and from about the tenth day on high or low temperatures only slightly inhibited development.

Studying this same factor in the incubation of turkey eggs in a natural-draft incubator, Martin and Insko (1935) found that a temperature above 103° F. during the fourth week of incubation increased embryo mortality, especially on the twenty-fifth day, the usual peak point of mortality in that period.

Since it had been shown that high temperatures caused death of the embryo, Henderson (1939) thought that a study of different temperature schedules might indicate one that would give maximum growth and minimum mortality. In addition, such a study might reveal the possibility of a shorter incubation period. Using forced-draft incubators he obtained the results given in table 25.

TABLE 25

EFFECT OF VARIOUS TEMPERATURES UPON LENGTH OF INCUBATION PERIOD
AND HATCHABILITY IN A FORCED-DRAFT INCUBATOR

(Henderson, 1939)

Temperature	Time of hatch	Percentage hatch of fertile eggs
104° F. reduced gradually to 100° F. by 20th day	20 days, 6 hrs.	64
105° F. reduced gradually to 100° F. by 11th day	20 days, 8.5 hrs.	10
101° F. constantly	20 days, 6.6 hrs.	62
99°-99.5° F. for 16 days reduced to 97° F. by 19th day	21 days, 8 hrs.	78

This experiment showed that a reduction in hatching time was accompanied by an increase in embryonic mortality. Early high temperatures were not followed by immediate mortality as might have been expected, but death was delayed until near hatching time and the effect of high temperature was particularly apparent in the number of eggs left on the trays when the hatch was completed.

Studying the same factor with turkey eggs, Goodearl (1942) using a forced-draft incubator, found that a temperature of

99° F. gave higher hatchability than a temperature of 98° F. and that lowering the temperature to 98° F. retarded the hatch nearly 24 hours. The results obtained are shown in table 26.

TABLE 26

EFFECT OF INCUBATION TEMPERATURE ON HATCHING TIME IN A
FORCED-DRAFT INCUBATOR
(Goodearl, 1942)

Temperature	Percentage of poult hatching on		
	27th day	28th day	29th day
98° F.	6.3	50.0	43.7
99° F.	31.3	62.5	6.2

In addition, with the same relative humidity in all incubators, the percentage of loss in egg weight increased with each increase of incubation temperature.

The appearance of chicks or poult may be considerably affected by temperatures above normal either before or after hatching. With high temperatures the eggs lose too much water, and the chicks are smaller and less alert than normal chicks. Unless special care is given, such chicks will not start to eat and drink as soon as they should, and, as a result, their muscles lose resilience, and the chicks appear to dry up and soon die. Since overheating may occur after hatching, several times during the hatching period the hatched chicks should be removed from the hatching trays and placed in chick boxes.

Results of experimental work on high temperatures seem to be in general agreement. For example, Barott (1937) found that at a temperature of 99° or 100° F. in a forced-draft incubator large, fluffy, lively chicks were hatched but that at 102° F. the chicks were much smaller and lacked alertness. At the higher temperature, there were also many abnormalities, those most common being crooked toes and spraddled legs. The chicks with spraddled legs were unable to stand. Barott found also many chicks with crooked necks, the chicks apparently being unable to straighten them after hatching. At 100° F., however, there were practically no abnormalities; crooked necks never appeared, and crooked toes appeared only very infrequently.

Goodearl (1942) compared the mortality records of poult in-

cubated and hatched at different temperatures in natural-draft machines (table 27). The results indicated that turkeys hatched at lower incubator temperatures were stronger than those hatched at higher temperatures.

TABLE 27

EFFECT OF STILL-AIR INCUBATION TEMPERATURE ON MORTALITY OF
POULTS HATCHED
(Goodearl, 1942)

Incubation temperature (degrees Fahrenheit)	Per cent mortality of poults to 3 weeks
100	21.0
101	14.3
102	19.0
103	33.3
104	100.0
105	30.0

Some specific effects of high temperatures upon the embryos have been reported to be defective development of brain, eyes, and the head region in general (Alsop, 1919) and failure of the yolk sac to be enclosed within the abdomen (Saint-Loup, 1890). There are doubtless other specific high-temperature effects which have been observed but not reported in the literature. Among these is the arrested development of certain organs and structures of the embryo. More work on the effect of high temperatures in incubation is urgently needed.

Low Temperature Effects

An operating temperature below normal causes a slow or late hatch, characterized by embryos still alive at normal hatching time, few eggs pipped, and a low hatch as a result. Chicks or poult from such a hatch show varying amounts of mortality dependent directly on the lateness of the hatch.

As mentioned before, Barott (1937) found that at 96° F. nearly all embryos died in the shell. Ronanoff *et al.* (1938) reported that the normal incubation period was retarded more than 4 days by a low temperature of 91.1° F. They found also that either lowering or increasing the temperature within certain limits during the second week had less influence on the time of hatch than

similar changes made in the first week of incubation and that changes made in the third week produced insignificant effects.

Alsop (1919) found that temperatures ranging from 94° to 101° F. produced 67 per cent of abnormal embryos, of which 83 per cent were defective in the neural tube and the remainder in the brain region. With an incubation temperature of 91.4° F., Tirelli (1900) reported that abnormal mitoses were common in the cell divisions of embryos showing retarded development. Dareste (1865), using an incubation temperature of 86° F., found that no embryos survived beyond the fourth day of incubation and that arrested development of the head, incipient cyclopia, and duplicity of the heart were common malformations. Later, Dareste (1869) also reported that blastoderms without embryos resulted from relatively low incubation temperatures.

Cooling or Chilling

For many years daily cooling of incubating eggs was practiced. The tray or trays were removed from the incubator and allowed to stay outside the machine for a short time, or the incubator door was left open for a similar length of time. Perhaps the chief value of this practice was in the removal of carbon dioxide from the incubator. With improved methods of ventilating incubators, the need for cooling or extra ventilation of eggs has ceased.

In addition to such planned cooling, current interruptions may cause temporary or protracted cooling of the eggs in electrical incubation. The outstanding work on this subject is that of Taylor, Gunns, and Moses (1933). These investigators selected a 12-hour period as representing the probable extreme limit of current interruption occurring in usual hatchery operations. Trays of eggs were removed each day to a chilling compartment, where the normal temperature was maintained for 1 hour; then the current was turned off, and the eggs left undisturbed until the temperature was restored after the 12-hour period. The results failed to indicate any particularly critical point at which current interruption decreased hatchability. This finding was not in agreement with an earlier report by Kaestner (1895) which indicated two periods of sensitivity, at 36 hours and at 6 to 7 days of incubation, respectively. Taylor *et al.* found that the average number of chicks produced in the chilled lots was 96.6 per cent

of those produced in the check lots. In the chilled lots there were more weak chicks, many having defective closure of the abdomen after the yolk sac had been taken up. There was little difference between chilled and check lots in the number of deformed chicks produced. The authors found no specific type of embryonic mortality caused by chilling but found that the increased loss was that of weak though normal-appearing embryos. The increased loss due to chilling was expressed in an increased proportion of deaths at the major peak in the embryonic mortality curve. Kaufmann (1934) used much lower chilling temperatures and found that embryos were more sensitive as incubation progressed.

Since current interruptions may cause considerable trouble, as these investigators have shown, a source of power, such as a stand-by generator of electricity, is good insurance. A prolonged interruption of current may be very disastrous, and thus the expense of the stand-by generator can readily be justified. If stand-by power is not available, special precautions should be taken with forced-draft machines while the current is off. Since the fans are not running, heat will increase especially near the top of the machine, and the carbon dioxide content of the air will be detrimental to the eggs at later stages of incubation. The doors should be opened at intervals of 15 minutes and fanned back and forth to get fresh air into the incubator. The incubator room should be warmed, if possible, above the usual temperature so that the air going into the incubator will not decrease the temperature further. In sectional incubators this ventilation is not necessary, since natural ventilation will suffice. Containers of hot water may be put in each section to help keep up the temperature.

If it is possible to keep the temperature during the interruption as high as 90° F., there will be only a slight delay in the hatch, usually about the length of time of the current interruption.

HUMIDITY

Optimum Conditions of Humidity

The proper level of humidity in the incubator is just as important for high hatchability as proper temperature. However,

if the humidity is either too high or too low the result is not so disastrous to the hatch as a prolonged increase in temperature above optimum. Proper levels of humidity induce improved bone formation and increased size of embryo. Levels below optimum result in less assimilation of bone-forming material and in smaller embryos. High moisture is particularly necessary at hatching time; low moisture at this time may be dangerous. The proper amount of moisture is necessary also during the earlier incubation period so that the embryos may grow properly.

There is widespread fear, usually groundless, that levels of humidity above the optimum will cause the chicks or poult to "drown" in the shell. It is true that chicks or poult produced under optimum conditions of humidity do not dry off quite so quickly as those produced under lower levels and are not so active when they are taken from the incubator. However, when they are allowed to dry for a few hours they are the equal of those produced under lower moisture conditions, and in addition they withstand shipping much better. If low-moisture conditions are allowed to persist throughout the hatching period, the chicks stick in the shell.

More moisture is required during the incubation of turkey eggs than during the incubation of chicken eggs. It should be noted that the temperature for incubation remains the same for each but that humidity should be higher for turkey eggs during the incubating period (24 days).

Approximately 60 per cent relative humidity gives excellent results with chicken eggs, and slightly higher humidity, 61 or 63 per cent, gives excellent results with turkey eggs. In practical operation this means that an incubator operating at a dry-bulb temperature of 99.5° F. should be operated at a wet-bulb temperature of 86° F. for chicken eggs and of 87° to 88° F. for turkey eggs. It should be noted also that, when moisture is raised during the hatching period, the temperature is often lowered, especially in a separate hatching compartment or separate hatcher. Again the manufacturer's directions should be followed rather closely. It should be pointed out that the moisture in the incubator should be raised at the time the eggs are transferred to the hatching compartment, on the eighteenth day for chicken eggs and on the twenty-fourth day for turkey eggs. The raising of the humidity

should be positive; that is, it should never be delayed until the first eggs pip; such a delay results in a higher mortality among those chicks or poultts pipping the shell early, because many of them will stick in the shell and will be unable to get out. When the eggs start to pip, the humidity will go even higher, but this effect is normal and usually offers no problem. Should the moisture become unusually high it may be reduced to 90° or 92° F. on a wet-bulb thermometer.

Effects of High and Low Humidity

Investigators are not in complete agreement on the effect of changes in humidity levels. Romanoff (1930) found that high humidity (80 per cent) hastened the growth of the embryo, whereas low humidity (40 per cent) retarded growth. High humidity increased calcium deposition in bones during the later stages of incubation. Mortality in the embryo was noticeably increased by high humidity during the last week of incubation. The optimum humidity level was found to be 60 per cent.

Results secured by Penquite (1938) are not in entire agreement with those just cited. With relative humidities of 40 per cent, 62 per cent, and 80 per cent, he found that there was no significant difference in the growth of chick embryos as measured by either wet weight or dry weight. It was found, however, that a relative humidity of 62 per cent gave best hatching results.

Townsley (1930a), using a series of forced-draft incubators, found that relatively high humidity is necessary for maximum results in producing liveable chicks and that as the humidity is increased the temperature requirement is reduced. Operating the incubator at 99° F., the wet-bulb temperature was maintained at 75°, 85°, and 90° F. in different machines. The results in percentage of hatch, average weight of chicks, and livability of chicks were in favor of the 85° F. wet-bulb temperature. Reducing the operating temperature without changing the wet-bulb reading in the high humidity machine gave a marked improvement in percentage of hatch, size of chicks, and their livability.

Townsley stated: "It appears that as humidity is increased the operating temperature must be decreased and when humidity is low the temperature must be run higher. . . . It seems evident from the above tests that a combination of high temperature and

high humidity will cause poor results in hatching while there is practically no danger of getting too much humidity in the incubator if the temperature is reduced in the proper ratio."

Barott (1937) conducted two series of experiments on humidity requirements. In the first series the temperature was maintained at 102° F., the oxygen content at 21 per cent, the carbon dioxide below 0.5 per cent, and the air movement at 12 centimeters per minute. The best hatches were obtained at 58 per cent relative humidity at this temperature. In the second series, conducted at 100° F., 61 per cent humidity gave the best hatches. The hatches decreased as the humidity varied in either direction. This decrease, at first slow, became more rapid as the humidity variation increased. Barott concluded that ". . . fairly satisfactory hatches may be expected within the range of humidity from 40 to 70 per cent, but at higher or lower humidities, small hatches of poor-quality chicks are bound to occur."

Pringle and Barott (1937) found that loss of weight of fertile eggs during incubation depends principally upon the relative humidity in the incubator. Weight losses decrease in direct proportion to the increase in humidity. An increase of 1 per cent in humidity causes the weight loss to decrease 0.01 per cent per day. Increasing the temperature from 96° to 99° F. caused only a slight increase in weight loss but an increase from 99° to 103.5° F. caused a pronounced *increase in loss of weight*. The loss became progressively greater with each degree of temperature until at 103.5° F. the loss was one-third greater than at 99° F. Daily loss of weight increased as incubation proceeded.

North (1941) studied the effects of humidity at high altitudes. He found that humidity at such altitudes should be higher than levels customary for low elevations and that it should be raised before the chicks pip the shell. Eggs allowed to remain at 60 per cent relative humidity gave 84 per cent hatchability. When humidity was raised gradually to 75 per cent during the peak of the hatch, there was an 89 per cent hatch. When humidity was raised to 75 per cent before the first egg pipped, the hatchability was 92 per cent. Increased moisture helped prevent sticky chicks and aided pipping.

Working with turkey eggs in a forced-draft incubator, Insko,

MacLaury, and Ringrose (1942) found that, with a constant dry-bulb temperature, a wet-bulb reading of 83° to 84° F. gave significantly poorer hatches than did 85° to 86° F. or 87° to 88° F. Eggs incubated at 87° to 88° F. wet-bulb for 24 days gave higher hatchability than those incubated at 85° to 86° F., in all hatches. The wet-bulb temperature was raised to 90° F. on the twenty-fourth day.

Since the higher level of humidity gave higher hatches throughout, it seemed that the optimum level might not have been reached. For this reason, another experiment was conducted, in which a wet-bulb temperature of 87° to 88° F. was compared with a wet-bulb temperature of 89° to 90° F. The lower humidity gave consistently higher hatches than the higher humidity indicating that optimum conditions had been reached in the first series of experiments. Poult from all these hatches were brooded, and they showed no significant differences in mortality.

Recommendations for Control of Humidity

The optimum levels of humidity are given in table 28. The following precautions regarding humidity may be helpful.

SECTIONAL INCUBATORS.

1. Never set eggs unless the moisture in the incubator is approximately sufficient in the compartment.
2. Provide sufficient water-pan surface to keep the moisture at the desired level.
3. At hatching time provide additional moisture by increasing the water-pan surface, by adding sponges or other objects which increase the evaporating surface, or, if necessary, use a spray to add additional moisture at frequent intervals.

4. If chicks pip the shell and cannot get out, add additional moisture even if the moisture is already high.

FORCED-DRAFT INCUBATORS. Operate the incubator as shown in table 28. Do not increase the moisture until the eggs are transferred to the hatching trays, but raise it at that time. *Do not wait for the increase in moisture which comes when eggs pip*, since this procedure causes mortality of embryos in the eggs that pip early because many of the chicks will stick in the shell.

TABLE 28

OPTIMUM LEVELS OF HUMIDITY

Type of incubator	Period (days)	Wet bulb (degrees)	Dry bulb (degrees)	Approximate relative humidity (per cent)
<i>Chicken eggs</i>	1-18	88-89	101-1st week 102-2nd week	58-60
	18-21	90-94	103-3rd week	60-71
	1-18	82-87 *	99-100	48-61
	18-21	90-92	99-100	70-73
<i>Turkey eggs</i>	18-21	90	96-98	73-78
	1-21	88-89	100.5-1st week 101.5-2nd week	61-65
	21-28	92-94	102.5-3rd week 103.0-4th week	65-71
	1-21	81-88 *	99-100	51-65
<i>Forced-draft</i> <i>(Combined)</i>	21-28	90-92	99-100	70-76
	21-28	90	96-98	73-79

* Note that the average humidity for the period in the forced-draft incubator will approximate the higher figure. The higher humidity should be used until the first eggs are placed in the hatching trays.

SEPARATE HATCHERS.

1. Raise the humidity to hatching level on the day the eggs are transferred (eighteenth day for chicken eggs, twenty-fourth day for turkey eggs).
2. When chicks are removed during the hatch, be certain the humidity returns to the proper level quickly.

the temperature needs to be somewhat above normal. This latter conclusion, however, may be open to question since the eggs will dry out faster than is normal and the tendency will be to produce small chicks and to lower hatchability.

Romanoff (1936) found that lowering the temperature in a forced-draft incubator by as much as 5.4° F. after the sixteenth day produced no ill effects. He further found that lowering the temperature was a safer procedure than risking a temperature increase above 99.5° F.

Since the humidity should be increased on the eighteenth day for chicken eggs and the twenty-fourth day for turkey eggs, these are also the most desirable times for lowering the temperature.

TURNING

Effect of Turning

Frequency of turning has a direct effect on the percentage of embryonic mortality. When eggs are turned only a few times during the day there will be higher early mortality than when they are turned more often. Turning corrects the tendency of the embryo to stick to the shell membranes if the egg is left in one position too long. In addition to the effect on the early peak of mortality, it has also been shown that turning tends to lower the final peak of mortality. This is surprising since the eggs are not being turned at the time of the last peak of mortality, and the exact cause of this effect is not known.

The most easily detectable effect of an increase in the number of times eggs are turned is higher hatchability. Clark (1933) found an increased growth of chicken embryos to be caused by an increase in the number of times the eggs are turned. He found, however, that this effect was lost after the tenth day of incubation. Insko and Martin (1933) reported that not only was there an increase in the hatchability of chicken eggs with more frequent turning but also, as the most apparent result, there was a decrease in early embryonic mortality and in the incidence of certain malpositions. The results secured are indicative of the improved conditions resulting when turning is increased (see tables 29 and 30).

TABLE 29

EFFECT OF TURNING PULLET EGGS TWO OR FOUR TIMES DAILY IN A
SECTIONAL INCUBATOR
(Insko and Martin, 1933)

Breed	Times turned daily	Total eggs	Per cent fertility	Per cent total eggs hatched	Per cent fertile eggs hatched
White Leghorn	2	481	90.6	74.4	82.1
	4	472	89.2	78.6	88.1
Barred Ply- mouth Rock	2	439	90.7	70.6	77.9
	4	421	91.0	74.1	81.5
Rhode Island Red	2	336	87.8	72.6	82.7
	4	327	89.9	78.0	86.7

TABLE 30

EFFECT OF FREQUENCY OF TURNING HENS' EGGS IN A
FORCED-DRAFT INCUBATOR
(Insko and Martin, 1933)

Breed	Times turned daily	Total eggs	Per cent fertility	Per cent total eggs hatched	Per cent fertile eggs hatched
White Leghorn	2	1032	87.1	58.7	67.4
	4	1004	88.0	62.0	70.4
	6	994	86.2	63.6	73.7
	8	968	85.2	66.5	78.1

Olsen and Byerly (1936) found that eggs might be turned as often as 96 times daily during incubation without detrimental results if they were rotated back and forth along their long axes or tilted up and down about their short axes. Eggs turned in this manner at intervals of 15 and 30 minutes hatched 6.8 and 7.0 per cent better than the controls which were incubated horizontally and turned by hand 3 times daily in a hit and miss fashion. When eggs were turned 96 times daily in only one direction and about their long axes, there was high embryonic mortality caused by ruptured blood vessels and broken yolk sacs. Marcacci (1889) had previously noted rupture of the vitelline membrane with certain types of turning.

A further important factor in turning, in addition to the num-

ber of times that eggs should be turned, is the time at which turning should be stopped. This point has been studied by several investigators. Byerly, Haynes, and Marsden (1938), using turkey eggs turned 3 times daily, studied three factors in the normal shift from incubator to hatcher. The shift was made on each of the following days: twelfth, fifteenth, eighteenth, twenty-first, twenty-fourth, and twenty-fifth days. The three factors studied were time of cessation of turning when the eggs were transferred; position—horizontal or at a 45° angle; and difference in temperature after transfer—99.75° F. or 97° F. Changes in these three factors prior to 21 days reduced hatchability about 3 per cent, a reduction which they found was due to failure to turn the eggs. They also found that longer-shaped eggs were more adversely affected by not being turned than shorter eggs.

Another experiment along the same line was that of Insko, MacLaury, and Ringrose (1942) who found no significant differences between hatchability of different groups of turkey eggs when the turning was stopped on the eighteenth, twentieth, twenty-second, or twenty-fourth day, provided that there was no increase in humidity until the twenty-fourth day. When eggs were transferred to hatching trays on the twenty-first and twenty-fourth days of incubation, with an increase in humidity on the day the eggs were transferred, a significant difference was found in favor of the twenty-fourth-day transfer.

Recommended Turning Practice

In the actual operation of the incubator it is good practice to keep a chart showing that the eggs were turned, the time of turning, and the direction in which they were turned. Such a chart provides a record by which to check later turning operations.

better and quicker to turn the eggs more often and save the time spent in leveling the eggs, and hatchability under this procedure has been found to be greater.

When automatic turning devices are used the eggs may be turned at regular intervals throughout the 24 hours. In hatcheries large enough to employ night men the eggs may be turned at equal intervals. If eggs are not turned during the night, however, it is best to turn them an uneven number of times so that they will not be on the same side for 2 nights in succession, since if one position is maintained for too long a time the embryo may tend to stick to the shell.

Some suggestions on turning procedures for both chicken and turkey eggs follow:

SECTIONAL INCUBATOR.

1. Turn at least 5 times daily.
2. Be certain that all eggs are completely turned. If they are not, regulate the method of turning to make a complete turn.
3. Do not smooth or level eggs. This method is too time consuming. If hatches are not so high as expected, increase the number of times turned rather than smooth the eggs.
4. Turn until the eighteenth or twenty-fourth days. No turning is required thereafter.

FORCED-DRAFT INCUBATOR.

1. Turn at least 5 times daily.
2. If an automatic turning device is available, turn 8 times or oftener.
3. Turn until the eggs are transferred to the hatcher on the eighteenth or twenty-fourth day. No turning is required thereafter.

POSITION OF EGG IN INCUBATOR

In sectional incubators eggs are allowed to lie on their sides with sufficient space between them to permit ease in turning. In forced-draft incubators eggs are usually placed on the small end and held either erect, large end up, or in a similar position but tilted at an angle of approximately 45° . In either type of incubator, good results may be secured if proper attention is given to other factors, particularly turning.

Position of Egg and Its Relation to Embryonic Malpositions

Although certain effects due to egg position are now taken for granted, they were established beyond doubt only recently. For example, Byerly and Olsen (1931) found that eggs that had been incubated with the small end up gave low hatchability, because a large number of the eggs had embryos with the head in the small end of the egg. In this position, away from the air cell, it is very difficult for the chick to hatch.

These same workers (Byerly and Olsen, 1933) showed that chick embryos in the normal position have more than twice the chance of hatching than those with the head in the small end of the egg have. In addition to low hatchability, eggs incubated with the small end up had a high percentage of embryonic mortality during the first 2 weeks of incubation. Byerly and Olsen later found (1936) that when the small ends of the eggs were held down during incubation, there was a noticeable decrease in the incidence of two malpositions: that of head in the small end of the egg (Malposition II) and that of head away from the air cell (Malposition IV). For further discussion concerning these malpositions see chapter 7.

Hutt and Pilkey (1934) studied the incidence of malpositions in eggs incubated: (1) in tilted trays with the large end of the egg at an angle of 45° above the horizontal end and (2) in similar trays kept horizontal. The tilted eggs were turned in the usual way by rotating the tray through an angle of 90°. The others were turned by hand. Both groups were turned 2 or 3 times daily. It was found that the frequency of Malposition I (head between thighs) was twice as great in tilted as in horizontal eggs and that Malposition VI (beak over wing) was 25 per cent higher. On the other hand, Malposition II (head in small end) was more than twice as frequent, and Malposition IV (rotated from air cell) more than 3 times as frequent in horizontal as in tilted eggs.

Proximity to Dead or Infertile Eggs

For a long time there has been a belief that an infertile egg or an early dead embryo will cause mortality in eggs adjacent to it. This idea is probably a result of the observation that occasionally infertiles or early deads are in clusters in the tray. To determine the truth of this belief, MacLaury and Insko (1947) examined 10,510 eggs that had been numbered and checked as to position in the tray. Subsequently the mortality in eggs next to 658 infertile eggs and 1215 embryos dying in the setting trays was compared with that in other eggs selected at random. Since a greater mortality was found in eggs next to the random samples, no evidence supporting the belief was obtained.

CARBON DIOXIDE AND OXYGEN

Since carbon dioxide, which is harmful in large amounts, is given off by eggs as they develop, ventilation is very important. Increased ventilation, in addition to removing carbon dioxide, also introduces a large amount of oxygen into the incubator. In general, sectional incubators can be ventilated properly without resorting to special mechanical devices. Forced-draft incubators must depend, however, on rapid air movement and often on fan ventilation of the hatchery room.

Limits of Tolerance to Carbon Dioxide (CO_2)

A moderate amount of carbon dioxide in the incubator is necessary to stimulate embryo growth, but large amounts tend to depress growth, and extreme concentrations cause death. Oxygen in ordinary quantities promotes embryo growth.

As long ago as 1914, Lamson and Edmond studied the amount of carbon dioxide found under setting hens and in incubators. They found that chicken embryos could withstand rather wide variations in concentration of carbon dioxide, but that approximately 60 parts of carbon dioxide to 10,000 parts of air gave best results. With more than 150 parts of CO_2 per 10,000 parts of air, hatchability decreased, and with over 200 parts of CO_2 death occurred.

In general, experimental work on this subject is in close agree-

ment. Romanoff and Romanoff (1933) found that the growth of the embryo during the first few days of incubation was apparently stimulated by the presence of a moderate amount of carbon dioxide in the incubator. Carbon dioxide at a level of 0.4 per cent gave excellent results in embryonic development. When the carbon dioxide was increased to 1.0 per cent or higher, growth was depressed and mortality of the embryos increased. At a concentration of 10.0 per cent of carbon dioxide, all embryos died by the end of the seventh day, and at an even earlier age when the concentrations were higher.

Barott (1937) reported a series of experiments on the effect on hatchability of varying the carbon dioxide content in the incubator. In one series, carbon dioxide levels varied from 0.5 to 4.0 per cent. The carbon dioxide of each lot was constant during the incubation period. Other factors that were also kept constant were temperature, at 99° F.; relative humidity, at 60 per cent; oxygen, at 21 per cent; air movement, at 12 centimeters per minute past the eggs. Within the range of carbon dioxide levels used, an increase of 1 per cent of carbon dioxide decreased the hatch about 15 per cent. With a carbon dioxide content of 4 per cent, less than 25 per cent as many chicks were produced as were produced with 0.5 per cent carbon dioxide. When the carbon dioxide content was constant at 2 per cent, the hatch was more than 35 per cent less than with 0.5 per cent carbon dioxide.

In a second experiment, the carbon dioxide was allowed to increase in the incubator for some lots. The first lot was held at 0.5 per cent throughout the experiment; in the second carbon dioxide was allowed to accumulate to 5.5 per cent on the tenth day, after which it was held constant at that level; and in the third, it was allowed to accumulate until it had reached 10 per cent on the thirteenth day and thereafter was held constant. The other factors held constant were: temperature, 102° F.; relative humidity, 60 per cent; oxygen, 21 per cent; and air movement, 75 centimeters per minute past the eggs. It was found that the gradual increase to 5.5 per cent with the carbon dioxide held constant thereafter was not so harmful as when the carbon dioxide was held at 4.0 per cent throughout the experiment. When the carbon dioxide was allowed to increase to 10 per cent results were not so good as when the carbon dioxide was kept constant at

4.0 per cent. It should be remembered, however, as was pointed out in the section on temperature, that this latter experiment was conducted at 102° F., as compared with 99° F. in the former, and that there was a hatchability difference of nearly 10 per cent in favor of a temperature of 99° F.

Incubators are so designed that the ventilation will take proper care of the exhaustion of the carbon dioxide. The incubator rooms should be arranged so that there are no dead air spaces or pockets in the room. An incubator placed against the wall or in a corner of the room may give poor results because of lack of proper ventilation.

Oxygen Requirements

A statement by Barott (1937) best summarizes the practical aspects of the problem of the oxygen requirements of incubating eggs. He said: "In incubation it is impossible to have an excess of oxygen unless it is artificially supplied. It is very easy, however, to get a deficiency as the carbon dioxide eliminated is produced at the expense of the oxygen."

Arbuckle (1918) showed that the oxygen dropped as low as 14.4 per cent to 16.5 per cent by the twenty-first day in a natural-draft incubator. The addition of oxygen increased the comfort of the chicks in the incubator, preventing their panting, chirping, and running about the trays.

In a series of studies on oxygen requirements Barott obtained best hatches with 21 per cent of oxygen. A deficiency of 5 per cent of oxygen reduced the hatch from 81 to 55 per cent, whereas an excess of 25 per cent of oxygen was required to reduce the hatch the same amount. Between 30 and 50 per cent, as oxygen was increased 1 per cent, there was a corresponding decrease of 1 per cent in the hatch, and for each 1 per cent decrease below 21 per cent there was a decrease of 5 per cent in the hatch.

Studying this problem further, Cruz and Romanoff (1944) exposed eggs to high oxygen concentration for 5 days. They found that the early growth of the embryo was accelerated by exposure to oxygen concentrations above 21 per cent. The greatest growth was observed at an initial concentration of 31 to 41 per cent, and highest hatchability was observed at an oxygen

concentration of about 32 per cent. At higher than this percentage of oxygen there was a decrease in hatchability.

A marked retardation of embryonic growth at the 4-day stage was observed by Giacomini (1895), when the air pressure was reduced to 16–17 centimeters of mercury. Formation of the normal circulatory system was retarded. Reduced pressure is accompanied by oxygen deficiency, and when oxygen was added normal development ensued.

Under ordinary conditions best results can be secured by the usual methods of ventilation of the hatchery room and incubators. It should be remembered, however, that ventilation must be adequate since, as pointed out by Barott, carbon dioxide will be produced at the expense of oxygen.

At high altitudes, air exerts less atmospheric pressure than at lower elevations, and the oxygen content of the air also exerts a lower partial pressure. At high altitudes hatches are also much poorer than at lower altitudes, as shown in a survey conducted by North (1947) of hatcheries in Wyoming, Colorado, western Nebraska, Montana, and Utah (table 31). He found that serious

ability, flocks in high altitudes gave excellent results. After a few generations of selection it was possible to secure a strain which produced nearly as good hatchability as strains at low altitudes.

Further studies were made by Ells and Morris (1947) of Wyoming, who secured increased hatches of chicken and turkey eggs when additional oxygen was added to forced-draft incubators, the partial pressure produced by oxygen being thereby increased to a level approximating that at sea level. The increases secured were especially marked when the oxygen was added for chicken eggs for all 3 weeks, for the first and second weeks, and for the first and third weeks of incubation. The addition of oxygen during the second or third week only gave no increases in hatchability (table 32). When turkey eggs were

TABLE 32

EFFECT OF ADDITIONAL OXYGEN ON PERCENTAGE HATCHABILITY OF
CHICKEN EGGS AT HIGH ALTITUDES
(Ells and Morris, 1947)

Time O ₂ added	Eggs set	Good chicks	Percentage hatch fertile eggs
All 3 weeks	62	48	87.3
1st and 3rd weeks	87	66	86.8
1st and 2nd weeks	94	75	83.3
2nd week	92	61	74.4
3rd week	91	62	74.7
None	57	37	74.0

given additional oxygen for 28 days the hatch increased almost 40 per cent as compared with that of eggs incubated without additional oxygen. The average oxygen percentage where oxygen was added for all 3 weeks was 25.7. The control incubator had an average percentage of 20.2, which is normal for Laramie, Wyoming.

Although the experiments cited are excellent and helpful, this phase of incubation research has not yet received the attention it deserves. Continued interest and further experimentation would be desirable, especially in high-altitude incubation studies.

DISINFECTION AND FUMIGATION

In general, success in fumigation and disinfection of incubators depends upon ordinary principles of cleanliness. A thorough cleaning of the incubator, removal of all chick down and foreign matter, and a complete cleaning of all hatching trays and racks are necessary. For most effective disinfection, formaldehyde or fumigants containing formaldehyde should be used. If a spray of any type is used, the material should never come in direct contact with the eggs.

Effect Upon Embryos and Chicks

Bushnell, Payne, and Coon (1929), after extensive bacteriological tests, determined the effective dose of fumigant now recommended. Using a forced-draft incubator operated at a dry-bulb temperature of 100° F. and a wet-bulb of approximately 90° F., they recommended 35 cubic centimeters of formalin and 17.5 grams of potassium permanganate per 100 cubic feet as the minimum dosage for controlling pullorum disease.

In 1931, Marcellus, Gwatin, and Glover found that 48-hour and 72-hour embryos were especially subject to injury from high concentration of fumigant. They used 2.5 cubic centimeters of formalin and 1 gram of permanganate per cubic foot of incubator space for 1 hour. They also found that chicks at the pipping stage were not harmed by exposure for 1 hour to the same concentration. Chicks 3 to 5 hours old exposed to a concentration of 1.5 cubic centimeters of formalin and 1 gram of permanganate per cubic foot suffered high mortality.

Studying this problem further, Graham and Michael (1932a) used 35 cubic centimeters of formalin and 17.5 grams of permanganate per 100 cubic feet, without change of air in three fumigations of 3 hours each, 12 hours apart, in a forced-draft incubator during the hatching period. They concluded that hatching chicks are not seriously affected by a germicidal concentration of formaldehyde but that chicks at later stages are seriously affected. These workers (1932b; 1933) advised leaving the incubator closed for 3 hours following the introduction of the fumigant in order

to obtain maximum germicidal results. In order to kill persistent infection remaining from previous hatches, Graham and Michael (1934) recommended releasing a double amount of formalin on the day preceding the hatch.

In the sectional type of incubator, Murphy (1932) recommended the same concentration as given above for forced-draft incubators.

In general these investigators have demonstrated the effectiveness of formaldehyde fumigation in the control of pullorum disease. They found fumigation to be more effective in forced-draft incubators than in sectional incubators because in the sectional incubator the concentration of fumigant is not always the same in different regions of the incubator, owing to the tendency of formaldehyde to settle.

Accepting the foregoing results and other similar reports, Insko, Steele, and Hinton (1941) were concerned only with the effect of formaldehyde fumigation on the mortality of chick embryos. They found that the newly hatched chicks showed surprising tolerance to formaldehyde at a germicidal level (35 cubic centimeters of 40 per cent formalin and 17.5 grams of permanganate per 100 cubic feet); mortality at this concentration was very low. It was found that embryos are most susceptible during the early part of the incubation period, usually on the second or third day. A fumigant of three times the normal concentration produced increased mortality during this early stage. Mortality during the rest of the incubation period was very low, even with three or seven times the normal amount of fumigant. The hatched chicks withstood considerable exposure to formaldehyde until the chicks were dry. Chicks should be fumigated only when they are damp and the humidity is very high.

Precautions Necessary in Fumigation

1. The incubator and eggs should be clean and should otherwise conform to the best practices in sanitation.
2. Fumigation at high concentrations should not be made during the first 3 days of incubation because the embryos are then most susceptible to formaldehyde.
3. Eggs in the separate hatching compartments of an incubator should be fumigated on the eighteenth to twentieth days of incubation.

4. Eggs may be fumigated at the time of hatching, but fumigation should never be delayed until the chicks have become dry.

5. The formalin should be standard 40 per cent commercial grade. It should be stored in a well-stoppered bottle. (Warning: Do not permit formalin to come in direct contact with the hands for it may cause serious skin trouble. Wear rubber gloves when handling it.)

6. Potassium permanganate should be kept in a colored bottle or moisture-proof container to prevent oxidation or loss of strength.

7. Just before fumigation the humidity in the incubator should be raised to 92° to 94° F. wet-bulb reading. The fumigation should be performed at normal operating temperature.

8. Fumigation by the permanganate method (see fig. 79 B) requires the following items:

- (1) Measuring graduate or bottle for the formalin.
- (2) Small balances or standardized measure for the permanganate.
- (3) Large earthenware or enameled dish for combining formalin and permanganate. (A large enameled wash basin or cooking utensil may be used.)

9. Effective germicidal fumigation for *S. pullorum* by the permanganate method requires about 35 cubic centimeters of 40 per cent formalin and 17.5 grams of potassium permanganate per 100 cubic feet. Converted to the standard usually employed by hatcherymen, this proportion is equivalent to 1.2 fluid ounces of formalin and 0.6 ounce of permanganate per 100 cubic feet (table 33).

TABLE 33

APPROXIMATE CONVERSIONS OF WEIGHTS AND MEASURES FOR USE
IN FUMIGATION

Fluid measure	1 fluid ounce = 30 cubic centimeters (cc.)
	1 pint = 16 ounces = 473 cubic centimeters (cc.)
	1 cubic centimeter = 0.0381 fluid ounce
	1 liter = 1000 cubic centimeters = 33.81 fluid ounces or slightly more than 1 quart
Weights	1 ounce (avoirdupois) = 28 grams
	1 pound (avoirdupois) = 16 ounces = 454 grams
	1 gram = 15 grains = 0.035 ounce (avoirdupois)
	1000 grams = 2 pounds, 3 ounces, and 120 grains or about 2.2 pounds

lorum. Seventy to 100 cubic centimeters of formalin and 35 to 50 grams of permanganate per 100 cubic feet is the concentration recommended for effective control of mushy-chick disease.

The amount of mortality with three times normal fumigation strength is not serious and if necessary the treatment could be still more severe provided that the fumigation is done after the fourth day of incubation.

12. Fumigation by the cheesecloth method (see fig. 79 A) requires the following items:

- (1) Measuring graduate or bottle for the formalin.
- (2) Cheesecloth of appropriate size.
- (3) Small hooks, tacks, or rods for holding cloth in place.
- (4) Bucket or basin in which to immerse cheesecloth in the formalin.
- (5) Rubber gloves to be worn while handling the cheesecloth saturated with formalin. (*Warning:* Serious skin trouble may occur if formalin comes in direct contact with the hands.)

13. When the cheesecloth method is used, pieces of cheesecloth about 1-yard square should be immersed in a sufficient quantity of formalin to supply 20 cubic centimeters (0.7 fluid ounces) of formalin per 100 cubic feet in incubator space. The cloth should then be hung over rods near the fan and allowed to remain for 3 hours. This method requires approximately two-thirds the quantity of formalin needed in the permanganate method.

14. Treatment by either method should last not less than 1 hour or more than 3 hours.

15. If suitable measuring and weighing facilities are not available, the operator should consult his local pharmacist or photographer about the weighing or measuring of the needed materials.

16. The recommendations of the incubator manufacturer should be considered in fumigating with formaldehyde.

MECHANICAL SHOCKS

Mechanical disturbances, either before or after setting, may cause decreased hatchability. If eggs are handled too roughly before incubation the air cells may be jarred loose and displaced. Such handling reduces the hatch through a decrease in availability of oxygen to the developing embryo.

When eggs are shaken rather violently during incubation, Olsen and Byerly (1938) reported heavy mortality especially between the fourth and the thirteenth days.

Stiles and Watterson (1937) reported that when eggs were jarred for 1 minute out of each 15-minute interval between the

fourth and twelve hours of incubation the hatch was reduced from 45 per cent (for the controls) to less than 1 per cent. Disturbances in vitelline circulation and in development of the neural tube and optic and auditory vesicles were found in shaken embryos.

These results indicate that care should be used in handling hatching eggs both before and after setting.

CONCLUSION

The experimental results cited do not attempt to give an exhaustive historical review of all the experimental work on physical conditions in incubation, but rather to give an up-to-date summary. The fact that in a period of 20 years there has been an improvement in hatchability from approximately 65 per cent to 85 per cent of the total eggs set should not keep us from endeavoring to obtain a still higher hatchability. If recommendations from all the experimental work on feeding, breeding, and incubation procedure could be followed closely in breeding flocks and hatchery management, the hatchability of fertile eggs should average well above 90 per cent. The fact that they do not average so much is a challenge to everyone in the hatchery industry.

It has been particularly stated that much more work should be done on incubation requirements at high altitudes. It should further be emphasized that there is much need for experimental work in all sections of the country and under all climatic conditions.

There is still no satisfactory explanation for the mortality of chicks that die after pipping the shell, as a result of the openings being sealed over by liquids from within the egg. Partial explanations of this can be given but they are not entirely satisfactory. Humidity requirements, especially for turkey eggs, have not been fully determined in all sections of the country. Temperature requirements are better understood, and it is especially well established that temperature above normal will cause high mortality.

A good incubator operator likes his work and is willing to keep complete and adequate records on temperature, humidity, turning, and other factors that will help him to repeat good hatches.

or to find the cause of poor hatches. A good operator reads the manufacturer's directions on the incubator operation frequently, and he understands his incubator and why it is constructed as it is. He realizes that all incubators of a given make should be run alike but that there may be slight differences in the controls which he should know and which he should regulate accordingly. It is well to become acquainted with the particular incubator being operated. Check the controls and thermometer before the beginning of the season and frequently thereafter. Keep spare parts on hand to replace those that might possibly give trouble. In other words, keep attention centered on the operation of the machine, being willing to devote extra hours if necessary to the job. Apply the principles of successful incubation operation, and good results should follow.

PRACTICAL RECOMMENDATIONS SUMMARIZED

General Procedure

1. Study the incubator manufacturer's directions frequently since failure to observe proper rules of operation may result in poor hatches.
2. Check thermometers and electrical controls well before the beginning of each season. A clinical thermometer should be available for checking the accuracy of the incubator thermometers.
3. Rinse the wicks of the wet-bulb thermometers daily and wash them with soap and water once each week. Deposits that accumulate on dirty wicks cause faulty wet-bulb readings.
4. Keep complete records of incubator operation including wet-bulb and dry-bulb temperature, time and direction of turning, adjustments, and other pertinent observations. Such records may be helpful in determining possible causes of poor hatches or may make it possible to duplicate good hatching conditions.
5. Faulty incubation frequently results in weakened chicks or poult. Check all factors in incubation frequently and operate the machine at all times at optimum conditions.
6. Keep spare wicks, pilot lights, wafers, microswitches, and other spare parts on hand at all times.

Incubation of Chicken Eggs

1. When incubating chicken eggs in a forced-draft incubator, operate the machine at the temperature recommended by the manufacturer. Since incubators may vary in design, the experience passed on to you in the operating directions should give you best results.

2. In a sectional incubator with the bulb of the dry-bulb thermometer on a level with the top of the eggs, operate the machine at 101°, 102°, and 103° F. for the first, second, and third weeks respectively.

3. A relative humidity of 55 to 61 per cent during the first 18 days of incubation and an increase to 70 per cent during the last 4 days of incubation should give high hatchability. For example, an incubator operated at 99.5° F. dry-bulb temperature should have a wet-bulb temperature of 85° to 87° F., during the first 18 days and 90° F. wet-bulb temperature during the last 3 days of incubation.

4. In a sectional incubator, if the eggs are pipping but the chicks are sticking in the shell, increase the moisture by increasing the surface of the water pans, adding sponges or other such objects to increase the evaporating surface.

5. The increase in moisture should be definite and positive, the increase being made at the time the eggs are transferred. There may be losses if the moisture is not increased until pipping starts, since some of the chicks will stick in the shell.

6. Turn the eggs at least 5 times or oftener daily and, if there is no turning at night, be certain to turn the eggs an uneven number of times so that they will not be left on the same side 2 nights in succession.

7. When setting trays are transferred intact to the hatching compartment, it is not necessary to remove infertiles and early deads, but hatched chicks should be removed at least 3 times during the hatching period to keep them from smothering.

8. When chicks are removed, be certain that the moisture in the incubator returns to normal hatching humidity shortly thereafter, or losses from drying out may be heavy. Add additional moisture if necessary.

Incubation of Turkey Eggs

1. The recommendations of the incubator manufacturers should be studied frequently and followed closely.
2. When incubating turkey eggs in a forced-draft incubator, run the machine at the temperature that has given the best hatches with chicken eggs, or, in other words, the temperature recommended by the manufacturer.
3. In a sectional incubator, excellent hatches may be secured if the machine is run at a temperature of 100.5°, 101.5°, 102.5°, and 103° F. during the first, second, third, and fourth weeks, respectively. These temperatures should be maintained with the bulb of the thermometer even with the top of the eggs or 1 $\frac{1}{8}$ inches above the egg tray.
4. A relative humidity of 61 to 63 per cent during the first 24 days of incubation and an increase to 70 per cent during the last 4 days of incubation should give high hatchability. For example: an incubator operated at 99.5° F., dry-bulb temperature, should have an average wet-bulb temperature of 87° to 88° F. during the first 24 days and 90° F. wet-bulb temperature during the last 4 days of incubation. This procedure gives a wet, slow-drying poult, but such poult ship, live, and grow well.
5. Increase in moisture in sectional incubators may be obtained by increasing the surface of water, by introducing sponges or other such objects into the water pans to increase the evaporating area.
6. The increase in moisture should be positive, that is, a definite increase should be made at the time the eggs are transferred. If the moisture is not raised until the eggs start to pip, losses due to drying may increase.
7. Eggs should be turned 5 times or oftener daily, and, if there is no one to turn the eggs during the night hours, the eggs should be turned an uneven number of times so that they will not be on the same side each night.
8. It is not necessary to transfer eggs incubated in a forced-draft incubator to a natural-draft incubator in order to assure high hatchability. If the proper amount of moisture is maintained in the incubating unit, slightly higher hatchability will be obtained in the forced-draft incubator.

9. It is practical to stop turning after the twentieth day of incubation and to transfer the eggs to the hatching compartment, but moisture in the hatching compartment should not be raised until the twenty-fourth day.

10. Hatching turkey eggs large end up in a forced-draft incubator with the trays full does not increase the hatch. This method is practical, however, if time is taken to remove poult from the hatching trays frequently.

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CHAPTER 7

Genetics and Physiology of Embryonic Development

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HEREDITARY FACTORS IN THE PHYSIOLOGY OF DEVELOPMENT AND HATCHABILITY

INTRODUCTION

It has been the experience of all poultry breeders that, despite every possible improvement in management and nutrition of the flocks, in storage and handling of the eggs before incubation, and in control of environmental factors during incubation, an appreciable proportion of fertile eggs still fail to hatch. Moreover it has been frequently observed that, under as nearly identical management as possible, some lines or breeds exhibit a higher hatchability than others, and within lines individual hens show very different hatchability records. When differences in frequency of embryonic mortality occur although variations in environmental conditions, nutrition, and exposure to disease are reduced to the minimum, it must be assumed that some of these differences are inherent.

The hereditary constitution of an individual is determined by units of inheritance known as genes. These are located in pairs of chromosomes, which are structures of varying shapes present in the nuclei of all cells. One member of each chromosome pair is contributed by the male parent via the sperm cell; the other member by the female via the egg or ovum. The genes are also paired. Each gene has a definite location on a specific chromosome, and the other member of the gene pair occupies the corresponding locus on the complementary chromosome. Thus one complete set of hereditary factors, or genes, is contributed by the dam, and a complete complementary set is derived from the sire. There is one exception. In the male bird complementary chromosomes of one certain pair are known as sex chromosomes. The female bird, however, has a single sex chromosome which is inherited from her sire. (The reverse is true of man; the female has two sex chromosomes, the male only one.) Genes located in the sex chromosome are referred to as being sex-linked. All chromosomes other than the sex chromosomes are known as autosomes.

During the course of evolutionary development, changes or mutations have occurred in many genes. Thus some genes have come to have a number of different forms which are known as alleles. Normally, only two of these alleles, constituting one gene pair, can of course be present in a single individual. The various alleles of the genes are responsible for the genetic differences between the members of a given variety of animals or plants. Some alleles are more favorable than others; some are definitely harmful. When both members of a pair of genes are identical, the individual is said to be homozygous for that particular allele; the presence of a pair of unlike alleles is known as the heterozygous condition.

White Silkie fowl. If a different allele were to be substituted for one of these recessive genes, the bird would have colored instead of white plumage. The white feathers of the White Leghorn breed, on the other hand, are determined by a dominant gene at another locus on another chromosome.

Occasionally neither of two alleles is completely dominant; in heterozygous combination they produce an intermediate effect. For instance, there are two allelic genes, one of which in double dosage results in black plumage color, the other in a splashed-white feather pattern. When the gene pair is composed of these two alleles, neither is dominant, and the bird is blue (Blue Andalusian). When traits are determined by a heterozygous gene combination such as this, the birds never breed true. Segregation of the genes occurs. One quarter of the offspring inherit the allele for black from both parents, one quarter derive the splashed-white allele from both dam and sire, and one half the progeny inherit a different allele from each parent. The expected ratio for color pattern among the offspring is therefore 1 black : 2 blue : 1 splashed-white.

A single dose of some dominant genes produces one effect, and a double dosage has a stronger, or even a different effect. Creeper fowl exemplify this type of inheritance; they will be discussed under the section on lethal genes.

Since the female bird has only one sex chromosome and one set of sex-linked genes, all sex-linked genes are expressed in the female, regardless of whether they may be recessive in the male, with his two sex chromosomes. The factors for barred feathers and slow rate of feathering are both sex-linked dominant genes. The respective recessive alleles of these two genes determine nonbarring and rapid feathering.

Although some characters are controlled by a single pair of genes, two or more gene pairs may be involved either directly or indirectly. For example, two pairs of recessive genes are necessary to produce single combs. A dominant allele of one of these genes causes rose comb, a dominant allele of the other results in pea comb, and when dominant genes for both pea and rose comb are present, a walnut comb is formed. In addition, there are indications that the presence of still other genes may produce various modifications of the basic comb form (see Jull,

their first experiment, selection was based on plumage color primarily and on number of offspring secondarily. The parental generation was derived from unrelated lines of Rhode Island Reds. In following generations, full brother-sister matings were made. The inbred stock was eliminated in 4 years by the decline in hatchability of fertile eggs * from 67 per cent to 18 per cent. The hatchability obtained for each successive year of inbreeding is entered in table 34, together with summaries of the

TABLE 34

PERCENTAGE OF HATCHABILITY OF FERTILE EGGS FOR SUCCESSIVE GENERATIONS OF CLOSELY INBRED CHICKENS

Source	Breed	Mating	Percentage of hatchability in successive generations					
			<i>P</i> ₁	<i>F</i> ₁	<i>F</i> ₂	<i>F</i> ₃	<i>F</i> ₄	<i>F</i> ₅
Cole and Halpin (1916)	Rhode Island Red	Inbred	67	49	41	18	.	.
		Control	...	31	56	64
Dunn (1928)	White Leg- horn	Inbred	75.0	50.5	46.7	34.3	46.8	41.6
		Control	49.8	67.5	60.8	60.1	59.2
Jull (1929a)	Barred Plym- outh Rock Plymouth Rocks, crosses of varieties White Leg- horn	Inbred	76.2	42.6	44.4	23.2
		Inbred	67.5	55.5
		Inbred	82.5	61.5	51.9	48.6
		Inbred	82	81	62	67	73	69
Waters (1945)	White Leg- horn	Inbred	89.3	84.6	81.3	94.3
		Inbred (se- lected as parents)	79.8	83.3	53.3	73.5	80.5	85.9
		Inbred (un- selected sisters)	86.8	83.4	91.8	83.5	85.9	85.9
		Outbred	86.8	83.4	91.8	83.5	85.9	85.9

results of a number of other representative studies. In their second experiment Cole and Halpin selected for hatchability and vigorous chicks; inbreeding was of the same intensity. At first the results seemed promising, but Dunn (1928) reported that this inbreeding program, despite the method of selection, also terminated in failure.

Dunn (1923b, 1928) reported similar results in experiments

* Hatchability, as used in this chapter, refers to the percentage of *fertile* eggs that hatch, not the percentage of total eggs set.

1940, for references). Such modifying genes have been demonstrated for numerous other traits.

The importance of the genetic constitution of the individual is acknowledged in the choice of specific animals as breeders in all planned breeding programs. Selection for body conformation, color, vigor, and reproductive ability is practiced with a view of propagating these desirable characteristics. Where the inheritance of a given trait is understood and simple in its mechanism, that character often can be established, or fixed in the stock by appropriate methods of selection. However, the inheritance of most physiological functions is not simple; combinations of many genes may be directly as well as indirectly involved. Such complexity of inheritance undoubtedly applies to hatchability. Selection under these circumstances may require considerable trial and error, and the development of a consistently good line may take many generations of carefully planned matings. Furthermore, there is no way of determining what heterozygous recessive genes are present, and there is always the possibility that new combinations of the genes derived from the two parents may have unfavorable effects. As Cole and Halpin (1916) remarked about inbreeding, "The end result depends upon the effectiveness of conscious selection together with the chance results of the large amount of unconscious and uncontrolled selection which must occur."

INBREEDING AND OUTBREEDING

Inbreeding

Desirable traits can sometimes be quickly established in a stock by close inbreeding, that is, by mating brothers with sisters, dams with sons, or sires with their daughters. Matings between more distant relatives constitute a slower, less intense program of inbreeding. Continuous close inbreeding is usually avoided by poultry breeders, for they have found that the outcome is likely to be a decline in vigor, in egg production, in fertility, and in hatchability. The experience of most investigators has tended to support this finding, although successful inbreeding has been practiced in recent years.

CHICKENS. Cole and Halpin (1916, 1922) recorded some of the earliest investigations of close inbreeding in poultry. In

their first experiment, selection was based on plumage color primarily and on number of offspring secondarily. The parental generation was derived from unrelated lines of Rhode Island Reds. In following generations, full brother-sister matings were made. The inbred stock was eliminated in 4 years by the decline in hatchability of fertile eggs * from 67 per cent to 18 per cent. The hatchability obtained for each successive year of inbreeding is entered in table 34, together with summaries of the

TABLE 34

PERCENTAGE OF HATCHABILITY OF FERTILE EGGS FOR SUCCESSIVE GENERATIONS OF CLOSELY INBRED CHICKENS

Source	Breed	Mating	Percentage of hatchability in successive generations					
			P ₁	F ₁	F ₂	F ₃	F ₄	F ₅
Cole and Halpin (1916)	Rhode Island	Inbred	67	49	41	18
	Red	Control	31	56	64
Dunn (1928)	White Leg-horn	Inbred	75.0	50.5	46.7	34.3	46.8	41.5
		Control	49.8	67.5	60.8	60.1	59.2
Jull (1929a)	Barred Plymouth Rock	Inbred	76.2	42.6	44.4	23.2
	Plymouth Rocks, crosses of varieties	Inbred	67.5	55.5
	White Leg-horn	Inbred	82.5	61.5	51.9	48.6
		Inbred	82	81	62	67	73	69
Waters (1945)	White Leg-horn	Inbred	89.3	84.6	81.3	94.3
	Rhode Island Red	Inbred (selected as parents)	79.8	83.3	53.3	73.5	80.5	..
Knox (1946)		Inbred (unselected sisters)	86.8	85.4	91.8	83.5	85.9	..
		Outbred

results of a number of other representative studies. In their second experiment Cole and Halpin selected for hatchability and vigorous chicks; inbreeding was of the same intensity. At first the results seemed promising, but Dunn (1928) reported that this inbreeding program, despite the method of selection, also terminated in failure.

Dunn (1923b, 1928) reported similar results in experiments

* Hatchability, as used in this chapter, refers to the percentage of *fertile* eggs that hatch, not the percentage of total eggs set.

with several inbred lines of Single Comb White Leghorns. The foundation stock for all lines was already slightly inbred, of good vigor and excellent hatchability. Selection was based on size of progenies. Most matings were between brothers and sisters; a few were between parents and offspring. The decline in hatchability was so great that Dunn was unable to go beyond five successive generations of sib matings, and only one of his eight lines survived as long as that. However, in these experiments disease epidemics increased the difficulties of preserving the inbred stocks. Marked variations in the rate of decline in hatchability within the several lines were good evidence of the segregation of inherent differences between the families. For example, Family IV with an initial hatchability of 70.4 per cent had a hatchability of 41.5 per cent in the F_5 (i.e., fifth filial) generation, whereas Family II with 95.8 per cent hatchability in the P_1 (parental) generation declined to 18.2 per cent in the F_4 generation, and Family VIII decreased from 85.2 per cent to 19.1 per cent by the F_3 generation. Dunn (1924) reported also that the various inbred lines differed in the developmental stage at which peaks of mortality occurred. For example, in one line almost half the deaths occurred before the sixth day of incubation, whereas in another family only 8 per cent of the mortality fell within this period. The hatchability results for all families are summarized in table 34. The increase in generation F_4 is due to the elimination of the six lines with the most rapid decline and is based on the two surviving families with the best hatchability records. Development appeared to be retarded by inbreeding; in the third generation, hatching was not completed until the twenty-second or twenty-third day. Dunn concluded that bad gene combinations had been made, but he suggested that inbreeding might be successful if the right foundation strains could be obtained.

Similar variations in the response to inbreeding of different White Leghorn lines led Goodale (1927), also, to suggest that eventually it should be possible to establish inbred lines genetically pure for various production characters. He stressed the necessity of selecting primarily for high hatchability and viable chicks if consecutive generations of sib matings are to be continued.

A decline in hatchability was also reported by Jull (1929a, 1933) as the result of inbreeding Barred Plymouth Rocks, Single Comb White Leghorns, and the progeny of reciprocal crosses between Buff and White Plymouth Rocks, and between Partridge and Silver Penciled Plymouth Rocks. Selection was based on large family size. Full-brother-sister matings gave lower hatchability records than half-brother-sister matings. The greatest increase in embryo mortality was at the end of incubation. Some of the hatchability data are summarized in table 34. Jull (1929b) further demonstrated for both the Barred Plymouth Rock and the White Leghorn stocks that the percentage of hatchability was inversely related to the coefficient of inbreeding—a mathematical estimate of the degree of inbreeding (Wright, 1923); that is, hatchability decreased as the degree of inbreeding increased (see table 35). According to Jull, the greatest decline in hatchability

TABLE 35

PERCENTAGE OF HATCHABILITY IN RELATION TO THE COEFFICIENT OF INBREEDING IN CHICKENS

(Adapted from Jull, 1929b, and Knox, 1946)

Source	Breed	Percentage of hatchability at inbreeding coefficient of					
		0	12.5	25.0-	37.5-	50.0-	60.4
Jull	Barred Plymouth Rock	76.1	47.3	40.1	21.0		
	White Leghorn	82.0	58.4	56.6	52.0	43.4
Knox	Rhode Island Red (selected as parents)	89.3	84.6	81.3	91.3	..
	Rhode Island Red (unselected sisters)	79.8	83.3	53.3	73.5	80.5

occurred in the first year of close inbreeding, and more or less consistent but lesser decreases took place in succeeding inbred generations. Knox (1946) obtained different results. They will be discussed below.

72 per cent to 33.5 per cent. Close inbreeding was found to be more deleterious than moderate inbreeding. In 1938 the same author reported that some inbred matings produced no loss in hatchability, whereas in other close matings there was a marked deterioration.

Experiments conducted by the British Northern Sub-Committee of the National Poultry Institute (Dunkerly, 1931; Northern Sub-Committee of the National Poultry Institute, 1934) also indicated that inbreeding reduced hatchability despite selection of breeders for various characters of vigor. White Wyandotte, Rhode Island Red, and White Leghorn fowl were used for two types of inbreeding experiments. In the first, males were bred to unrelated pullets, and in the following year, to their selected daughters out of these pullets; in the second, pullets out of brother-sister matings were backcrossed to their own fathers. In short, the first experiment involved one generation and the second, two generations of inbred matings. In each experiment, as a control cross, the same males were mated to unrelated pullets. The average hatchability was highest for each breed in the control cross, and two generations of inbreeding resulted in the lowest hatchability.

Hall (1934), who reported the results of selection for high- and low-egg-producing lines during fifteen years of matings involving a low degree of inbreeding with White Leghorns, found that the level of hatchability was subject to considerable annual variation; however, there was no apparent trend towards either an increase or a decrease in hatchability.

Hays (1924, 1934), Warren (1934), Byerly, Knox and Jull (1934), Veechi (1936), Borissenko (1945) and Bernier (1947) all confirmed the results of earlier investigators, finding in general that inbreeding in chickens increased embryo mortality, and the more intense the inbreeding, the more disastrous the effects on hatchability. Incidental data on hatchability from various other investigations not directly concerned with this problem also tend to support the same conclusion. Physical changes in the eggs from inbred White Leghorn fowl were shown to parallel a decline in hatchability, but no causal relationship between the two phenomena could be established (Borissenko, 1945).

Encouraging results from a ten-year study on inbreeding White Leghorn fowl at the Iowa Agricultural Experiment Station were reported by Waters and Lambert (1936a, b). They started with an excellent foundation stock; and rigid selection for high hatchability, as well as for vigor, size of family, and various other desirable characters, was continued throughout the experiment. A number of different successful family lines were developed, each

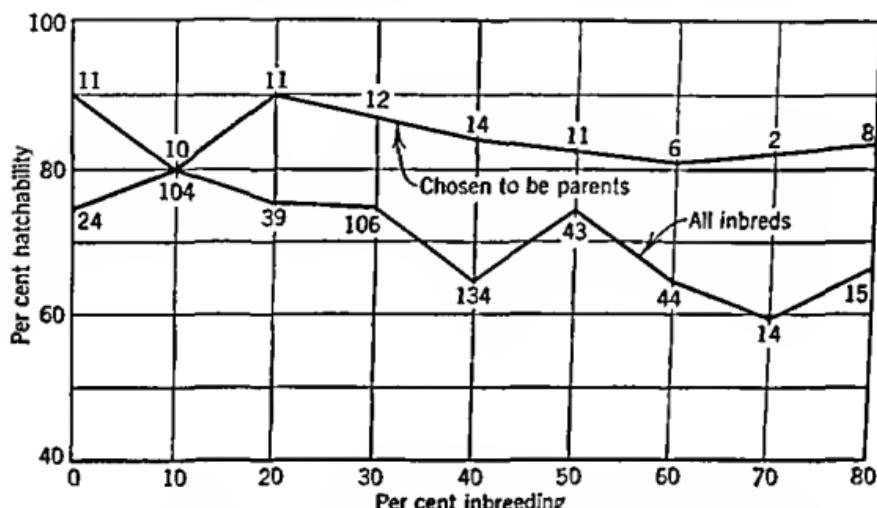


FIG. 51. Trends showing average per cent of fertile eggs hatched for all inbreds and for those inbreds chosen to be parents, together with the number of birds in each group. (After Waters and Lambert, 1936a.)

of them descended for the most part from the same few individuals. The degree of inbreeding attained in different lines varied, being generally less intense than would have been obtained by full-brother-sister matings in every generation. In one family, however, the equivalent of nine generations of sib matings was successfully attained without an excessive decline in hatchability. Although in many lines a slow and gradual decrease in hatchability occurred as the intensity of inbreeding increased, in other families there was no significant change. In fact, the average hatchability of all inbred lines remained above 59 per cent, and that of the chosen parents was maintained above 80 per cent (see fig. 51). As other studies had also indicated, upon continued inbreeding the various families diverged with respect to average

hatchability and numerous other traits. Waters and Lambert demonstrated conclusively that, with a good foundation stock, rigid selection, and a moderate rate of inbreeding, a high average hatchability can be successfully maintained in chickens.

From a study of fifteen additional inbred lines of White Leghorn fowl, Waters (1945) corroborated the earlier findings. A general decline in hatchability—which, however, did not fall below 60 per cent—was attributed, at least in part, to four distinct lethal genes, one to three of which were present in each line. Hatchability improved again after the elimination of some of these lethals. Waters suggested that inbreeding should not be too intense at the beginning of the breeding program in order to allow for the elimination of undesirable recessive genes before they can become widespread in the population.

Knox (1946) likewise recommended careful selection of the parent stock and of all subsequent generations and the maintenance of a low coefficient of inbreeding during the early stages of an inbreeding program. He demonstrated successful inbreeding without a decline in hatchability or losses in other desired characters. For one family of Rhode Island Reds in which the first generation of parents (P_1) had a hatchability of 89 per cent, the average hatchability for all pullets of the F_4 generation was 80.5 per cent. Table 34 gives the hatching records of the selected breeders in each generation, the average hatchability for all sisters of the female chosen as the dam in each generation, and, for comparison, the average from a flock of outbred Rhode Island Reds. Table 35 gives these data in terms of coefficients of inbreeding. After four or five generations of such inbreeding with selection. Knox found that fertility and hatchability levels could be "fixed" in an inbred line to a considerable extent.

TURKEYS. There has been relatively little pedigree breeding of turkeys, and very few data on the effects of inbreeding are available. It is assumed, however, that most of the conclusions drawn from chicken breeding apply to turkey breeding as well. Inbreeding is generally considered undesirable.

Marsden and Knox (1937) reported the results of a five-year turkey-breeding project, in which the Bronze variety was used, by the United States Bureau of Animal Industry. It was found that hatchability decreased as the intensity of inbreeding in-

creased. Outbred turkeys had an average hatchability of 67.6 per cent. With inbreeding coefficients of 12.5-41.1 hatchability dropped to 52-53 per cent; intensive inbreeding (coefficients of 50.0-67.2) resulted in a further decrease to 34.9 per cent. However, since hatchability varied greatly in various matings, with some inbreds giving better results than some of the noninbred controls, the authors suggested that it might be possible to develop good inbred lines by careful selection.

According to Asmundson and Jukes (1939), experiments at the California Agricultural Experiment Station also showed that close inbreeding of turkeys results in a lowered hatchability. Although it was found that different strains varied in their response to continued close inbreeding, it was concluded that inbreeding should generally be avoided.

On the other hand, Clark, Runnels, and Livesay (1944) have reported hatchabilities higher than 70 per cent for two generations of brother-sister matings in a small group of Black turkeys.

The possibility of maintaining a satisfactory level of hatchability in turkey inbreeding was also demonstrated by Jull and Phillips (1946). Successful sib and parent-offspring matings were achieved by the selection of the breeding stock on the basis of the average hatchability of the dam's family. In one mating between unrelated Beltsville Small White turkeys, the hatchability was 92 per cent. The following year 16 young hens out of this mating, when backcrossed to their sire, gave an average hatchability of 86 per cent; the difference is not significant. Only 4 of these 16 young hens had hatchabilities below 85 per cent, the lowest being 67 per cent. In another mating, 3 sisters bred to their full brother gave an average hatchability of 82 per cent.

Outbreeding: Heterosis and Hybrid Vigor

Various types of outbreeding have been practiced as a means of improving poultry stocks by the well-known phenomenon of hybrid vigor, or heterosis. Outcrossing is the term generally used to describe matings of unrelated members of the same breed or variety, whereas crossbreeding refers to matings between individuals of different breeds or varieties. If the birds used for such a hybrid cross are derived from inbred lines or families, incrossing is used to denote crosses between different lines within a breed,

and incrossbreeding for crosses between breeds. Topincrossing describes matings in which inbred males are crossed with unrelated noninbred females. Where cross matings (as well as standard-bred matings) produce exceptionally superior offspring, the combination is said to have "nicked."

The good results frequently derived from outbreeding are due to favorable new combinations of genes in the hybrid offspring. Whereas the progeny of an inbred mating inherit many pairs of identical genes and tend to become homozygous for bad as well as good traits, the progeny of an outbred mating derive a more or less different set of hereditary factors from each parent. Most deleterious genes are recessive, and it is not likely that unrelated parents contribute identical unfavorable recessive genes. The hereditary contribution from one parent tends to prevent the expression of undesirable genes derived from the other parent and vice versa, with the result that, to a certain extent, the best features from each parent are expressed and the worst features suppressed in the hybrid progeny.

Superior hatchability from outbred as compared with inbred matings has already been indicated in the preceding pages. It is true, nevertheless, that the results of outbreeding are very unpredictable in general. The good traits which are determined by homozygous genes in the purebred parental lines may be lost, and the progeny of the hybrids will not retain whatever hybrid vigor their parents may have possessed.

CHICKENS—CROSSES BETWEEN NONINBRED LINES. Pearl and Surface demonstrated the improved hatchability of hybrid eggs as early as 1910. Using a Barred Plymouth Rock strain with an average hatchability of 47.7 per cent and a purebred Cornish Indian Game strain with 55 per cent average hatchability, they obtained hatchabilities ranging from 66 per cent to 73 per cent in reciprocal crosses. The data from this report and several others are summarized in table 36.

Warren (1927, 1931) crossed the Jersey Black Giant and Single Comb White Leghorn breeds and obtained increased hatchability not only in the initial cross but also in the following F_1 inter se mating (see table 36). The improvement in the first mating was attributed to the vigor of the hybrid embryo, and in the second to the vigor of some of these same hybrids as dams. Warren (1930,

TABLE 36

HETEROSIS IN CHICKENS AS MEASURED BY HATCHABILITY OF EGGS FROM
PUREBRED AND CROSSED MATINGS

(Adapted from sources given)

Source	Mating		Number of fertile eggs	Percentage hatched
	♂	♀		
Pearl and Surface (1910)	BPR	× BPR	...	47.7
	CIG	× CIG	376	55.0
	CIG	× BPR	427	66.0
	BPR	× CIG	343	73.0
Warren (1927)	WL	× WL	5546 *	73.2
	JBG	× JBG	755 *	57.2
	{ JBG × WL }		284 *	77.8
	{ WL × JBG }		611 *	80.6
Funk (1934)	BPR	× BPR	435 *	57.0
	RIR	× BPR	202 *	74.0
	WL	× WL	1239 *	70.6
	WPR	× WL	266 *	80.1
Bice and Tower (1939)	JSG	× JSG	41	58.5
	BPR	× BPR	302	55.3
	JSG	× BPR	372	63.2
	WL	× WL	500	53.6
Horlacher, Smith, and Wiley (1941)	JSG	× WL	501	65.7
	RIR	× RIR	227	67.0
	JSG	× RIR	413	62.7
	RIR	× RIR	336	76.8
Warren (1942)	WW	× WW	257	79.4
	WW	× RIR	380	84.2
	RIR	× WW	80	82.5
	WL	× WL	463	65.7
Warren (1942)	WW	× WL	70	00.0
	RIW	× RIW	120	93.3
	WW	× RIW	179	89.0
	WR	× WR	197	72.6
Warren (1942)	WW	× WR	145	62.8
	WR	× (RIR-WW)	65	81.5
	BR	× BR	193	79.3
	WR	× BR	210	80.1
Warren (1942)	WL _w	× WL _w	289	61
	BPR	× WL _w	406	80
	WL _w	× WL _e	167	84
	BPR	× WL _e	387	86
Warren (1942)	WL _e	× WL _e	132	71
	BPR	× WL _e	584	80
	WL	× WL	199	73
	Aus	× Aus	199	72
Warren (1942)	Aus	× WL	231	81
	WL	× Aus	114	90

Aus = Australorp

BR, BPR = Barred Plymouth Rock

CIG = Cornish Indian Game

JBG = Jersey Black Giant

JSG = Japanese Shamo Game

RIR = Rhode Island Red

RIW = Rhode Island White

WL = White Leghorn (subscripts refer to different strains)

WPR, WR = White Plymouth Rock

WW = White Wyandotte

* Total number of eggs set.

1934) also found that crosses between different strains of White Leghorns yielded good results, which were, however, less outstanding than those obtained by crosses between breeds. The same investigator (1942) later reported on more than 20 different crosses involving 12 different breeds and varieties; in all tests except one, the hatchability of crossbred chicks was higher than that of comparable purebreds. There was considerable variation in hatchability in these crosses, just as different purebred strains vary in hatchability. It was observed that if one or both of the pure breeds involved had high levels of hatchability, there was less hybrid improvement. The results of reciprocal crosses were frequently quite different from each other. Representative excerpts from Warren's data are given in table 36.

Byerly, Knox, and Jull (1934) also found that crossbreeding improved hatchability in inverse proportion to the hatchabilities of the parental stocks. If the breeds crossed had hatching averages of 80 per cent or more, little improvement could be expected; actually at this level, crossbreeding resulted in a decrease in hatchability about as often as in an increase. These workers obtained an average hatchability of 69 per cent of 19,547 eggs from 11 pure breeds. The average hatchability of 12,999 eggs from 35 different crosses between pure breeds was 76 per cent.

Similar increases in hatchability from crossbreeding have been reported for most crosses by numerous other investigators. Funk (1934) found that hybrids between Rhode Island Reds and Barred Plymouth Rocks and between White Leghorns and White Plymouth Rocks hatched better than either the purebred Barred Plymouth Rock or White Leghorn stocks. Bice and Tower (1939) obtained improved hatchability from crossbreeding Japanese Shamo Game with Barred Plymouth Rock and Single Comb White Leghorn fowl. Hybrids from crossing the Japanese Shamo Game and Rhode Island Red breeds hatched better than the Game stock, but less well than the purebred Reds. Horlacher, Smith, and Wiley (1941) also found that most, but not all, crossbreds were superior in hatchability. Shoffner (1944) made reciprocal crosses between the White Leghorn, New Hampshire, and White Plymouth Rock varieties and obtained increased hatchability. Bernier (1947) observed that outcrossing between different strains of White Leghorn fowl produced greater improve-

ments in hatchability than did crossbreeding. Both types of hybrids hatched better than inbred chicks. Data from some of these reports are included in table 36.

Dudley (1944), on the other hand, found that reciprocal cross-mating between Rhode Island Red and White Leghorn fowl produced little if any heterosis with respect to hatchability. The breed of the dam appeared to be the important factor, with Rhode Island Red dams giving the higher hatchability. Byerly (1930) also had previously obtained more viable embryos from Rhode Island Red than from White Leghorn dams, regardless of the breed of the sire. However, in Byerly's experience, crossbreeding improved the hatchability from dams of both breeds. One expression of the improvement was a reduction in the frequency of teratological abnormalities (monsters) among the crossbred embryos. The data from these two reports are given in table 37.

TABLE 37

HATCHABILITY DIFFERENCES IN RECIPROCAL CROSSES OF RHODE ISLAND RED AND WHITE LEGHORN FOWL

Source	Mating		Number of fer- tile eggs	Percent- age hatched
	Male	Female		
Byerly (1930)	Rhode Island Red	× Rhode Island Red	1666	66.4
	White Leghorn	× Rhode Island Red	245	76.5
	White Leghorn	× White Leghorn	829	50.5
	Rhode Island Red	× White Leghorn	1303	56.2
Dudley (1944)	Rhode Island Red	× Rhode Island Red	71.9
	White Leghorn	× Rhode Island Red	74.0
	White Leghorn	× White Leghorn	66.1
	Rhode Island Red	× White Leghorn	66.5

Knox and Olsen (1938) and Knox (1939) found that crosses of either White Leghorn or Rhode Island Red fowl with a number of other breeds produced very variable results with regard to hatchability and to certain other traits. No really good combinations were obtained from these crosses. The authors concluded that the quality of the parental stock was a very important factor in determining the quality of the hybrid progeny. The data on hatchability indicate, however, that the average for cross-bred matings was slightly higher than that for various pure strains of White Leghorns and considerably higher than for at least one

strain of Rhode Island Reds. Knox, Quinn, and Godfrey (1943) found no improvement in hatchability from either two- or three-way crosses involving the Rhode Island Red, White Wyandotte, and the Light Sussex breeds.

CHICKENS—CROSSES BETWEEN INBRED LINES. The immediate favorable effect of crossing inbred strains of poultry (incrossing) was reported by Dunn (1928). In crosses between various inbred lines with very low hatchabilities, hatchability sometimes was nearly doubled. The improvements varied with the different lines crossed, indicating specific genetic differences between the various lines. Some of these results are given in table 38. Dumon (1931)

TABLE 38

THE EFFECT ON HATCHABILITY OF CROSSING INBRED-LINES OF CHICKENS

Source	Breed	Mating	Number of eggs	Per cent of fertile eggs hatched
Dunn (1928)	White Leghorn	Inbred-line 2	174	47.4
		Inbred-line 4	140	53.0
		Inbred 2 X Inbred 4	151	85.0
		Inbred-line 8	136	56.3
		Inbred 2 X Inbred 8	127	73.4
Jull (1930)	Barred Plymouth Rock	Inbred	...	23.1
		Intercrossed	...	43.8
	White Leghorn	Inbred	...	46.5
		Intercrossed	...	58.2
Knox (1946)	Rhode Island Red	Inbred	118	83.3
	R.I.R. X W.L.	Incrossbred	106	73.9
	Rhode Island Red	Inbred	81	53.3
	R.I.R. X W.L.	Incrossbred	158	74.0
	Rhode Island Red	Inbred	104	73.5
	R.I.R. X W.L.	Incrossbred	40	100.0
	Rhode Island Red	Inbred	254	80.5
	R.I.R. X W.L.	Incrossbred	113	90.7

also demonstrated a pronounced increase in hatchability when hens from his inbred lines were mated to cocks from other strains. Jull (1930, 1933) intercrossed lines of Barred Plymouth Rock and White Leghorn fowl that had been inbred for 3 years and obtained significant improvements in hatchability (see table 38). Hays (1934) likewise found that hatchability could be improved

in inbred lines of Rhode Island Red fowl by intercrossing the inbred lines. However, despite the improvement, the incrossed lines were in no respect superior to the general flock in Hays' experience.

Waters (1938) found the average hatchability of eggs from a topincross of inbred White Leghorn sires on random-bred females of the same breed to be 9.7 per cent higher than the average hatchability of the inbred fowl, and 5 per cent higher than that of random-bred flocks.

Knox (1946) suggested that, whereas outercrossing and crossbreeding of noninbred stocks yield very variable results in the hybrid progeny, it is possible to obtain fairly predictable results from crossing two different inbred lines. Since inbreeding tends to reduce variability to the minimum, repeated crosses between the same two inbred lines result in remarkably constant levels in hatchability and performance of the hybrid progeny. Knox pointed out that this is one of the most valuable uses for inbred lines. Some of his data are given in table 38.

TURKEYS. Heterosis, or hybrid vigor, has also been demonstrated in turkey breeding, but on a more limited scale. Clark, Runnels, and Livesay (1944) made reciprocal crosses between Standard Bronze, Bourbon Red, and Broad Breasted Bronze turkeys, and some three-way crosses involving the Black variety as well. They found a definite improvement in hatchability in most of the crosses, the average increase being approximately 8 per cent. The improvement may have been due in part to the low levels of hatchability of the parental stocks. Table 39 presents some of these results for purebred as compared with outercross and crossbred matings.

Jull and Phillips (1946), on the other hand, found no great difference in hatchability between outbred, moderately inbred, and crossbred matings.

Conclusions

Although most of the available data on the effects of inbreeding and outbreeding are derived from experiments with chickens, it appears likely from the few experiments in planned turkey breeding that similar conclusions apply to both chickens and turkeys.

TABLE 39

HATCHABILITY OF PUREBRED, OUTCROSSED, AND CROSSBRED TURKEYS
 (Adapted from Clark, Runnels, and Livesay, 1944)

Mating			Total number of eggs set	Per cent fertile eggs hatched
Male	Female	Type		
Bronze	Bronze	Purebred	258	67.1
	Bourbon Red	Crossbred	80	72.1
Bronze	Bronze	Purebred	181	71.8
	Bourbon Red	Crossbred	105	81.6
Bronze	Bronze	Purebred	272	62.9
	Bourbon Red	Crossbred	128	69.4
Bronze	Bronze	Purebred	255	62.7
	Bourbon Red	Crossbred	83	66.2
Bourbon Red	Bourbon Red	Purebred	185	70.2
	Bronze	Crossbred	74	64.6
Bourbon Red	Bourbon Red	Purebred	160	70.2
	Bronze	Crossbred	102	67.1
Bourbon Red	Bourbon Red	Purebred	221	72.5
	Bronze	Crossbred	110	79.3
Bourbon Red	Bourbon Red	Purebred	170	55.4
	Bronze	Crossbred	119	81.0
Bronze	Bronze	Purebred	174	56.8
	F ₁ (Black-B.R.)	Crossbred	112	79.6
Bronze	Bronze	Purebred	187	65.2
	F ₁ (Black-B.R.)	Crossbred	98	76.9
Bronze	Bronze	Inbred	213	47.6
B.B. Bronze	B.B. Bronze	Purebred	194	59.2
B.B. Bronze	B.B. Bronze	Purebred	114	53.3
B.B. Bronze	Bronze	Crossbred	219	67.4
Bourbon Red ₁	Bourbon Red ₂	Outcrossed	174	61.6
B.B. Bronze	Bourbon Red	Crossbred	279	75.6
Bronze ₁	Bronze ₂	Outcrossed	250	66.8
	B.B. Bronze	Crossbred	262	64.0
B.B. Bronze ₁	B.B. Bronze ₂	Outcrossed	260	49.3
	Bronze	Crossbred	220	65.8
B.B. Bronze ₁	B.B. Bronze ₂	Outcrossed	201	50.9
	Bourbon Red	Crossbred	267	58.9
Bourbon Red ₁	Bourbon Red ₂	Outcrossed	205	62.2
	B.B. Bronze	Crossbred	177	71.3
B.B. Bronze ₁	B.B. Bronze ₂	Outcrossed	219	60.4
	Black	Crossbred	186	85.5

Subscripts (1) and (2) indicate different strains in an outcross mating.

B.R. = Bourbon Red variety.

B.B. Bronze = Broad Breasted Bronze variety.

1. The quality of the foundation stocks and the selection of breeding birds for high hatchability and vigor are of primary importance in determining the effects of either inbreeding or outbreeding.

2. With good parental stocks and rigid selection, it is possible to develop fairly homogeneous inbred lines without sacrificing hatchability. However, since a high level of hatchability in the parent stock appears to be absolutely essential for any successful inbreeding program, an improvement in hatchability should not necessarily be expected among the inbred progeny. The establishment of good hatchability as a constant trait of an entire inbred line is in itself an important and, it now seems, a possible achievement.

3. In order to select against undesirable characters, inbreeding should not be too intense at the beginning of a program. After the elimination of birds that are proved to be carriers of unfavorable factors, closer inbreeding can proceed with greater safety. When undesirable characters cannot be eliminated, the inbred line must be discarded.

4. The results of outerossing or crossbreeding random-bred flocks are very unpredictable. Marked improvement in hatchability can often be obtained by crossing poor or mediocre strains. Contrarily, intercrossing superior foundation stocks results as often in a decreased as in an increased hatchability.

5. Remarkable constancy in hatchability and hybrid performance can be achieved by repeated crossing of the same two good inbred lines. This appears to be one of the most promising developments for commercial poultry production, since the producer can estimate the level of hatchability and the vigor and performance of the hybrid birds fairly accurately. It should be emphasized, however, that the results of crosses among the hybrid offspring are entirely unpredictable, as a complete reassortment of genes takes place in the mating of F_1 individuals.

LETHAL AND SEMILETHAL GENES

It has already been noted that one cause of poor hatchability may be the presence of unfavorable genes in the stock. Lethal genes cause the death of the embryo, usually at some particular

tibility of heterozygous embryos. Other lethal genes may have another type of effect in the heterozygote. One dose of the gene may produce morphological changes that are not fatal, and two doses may exaggerate the altered morphology to a fatal degree. This is true of the Creeper mutation which will be discussed below.

Recessive sex-linked lethal genes form a special class of lethals. In single dosage a sex-linked lethal has no effect on the male bird, as the second sex chromosome of the male carries a normal allele which prevents the expression of the lethal. But in the female embryo, a recessive lethal gene carried on the single sex chromosome acts like a dominant and is fully expressed. Therefore, the presence of sex-linked lethals can easily be detected by abnormal sex ratios. If the sire is heterozygous for a sex-linked lethal gene, all his sons, half of whom will inherit that lethal, will be normal, because they all derive a normal allele from the dam. Since the female bird inherits her one sex chromosome from the sire, half of the carrier sire's daughters inherit the lethal allele and die. The sex ratio of the progeny, then, is two males to one female instead of approximately one to one. Figure 52 illustrates the mode of inheritance of recessive sex-linked lethal genes.

In 1924 Dunn suggested that there might be numerous lethal and semilethal factors among poultry. At that time he had evidence of only one lethal gene in chickens. Since then eighteen more lethals have been demonstrated in chickens, and three have been found in turkeys.

Chickens

WYANDOTTE LETHAL. The first lethal gene found in chickens was discovered by Dunn (1923a) in the White Wyandotte variety. White color in this variety is determined by a homozygous recessive gene. When White Wyandottes are crossbred to colored varieties of fowl that do not carry this gene, the offspring are colored, but they carry one recessive gene for white. When these hybrid offspring are mated again to White Wyandottes (a backcross), all the progeny of the second mating inherit the gene for white from the white parent. Half of them also derive a white gene from the hybrid parent; the other half inherit the gene for colored plumage. Therefore, the expectation

stage of development, or cripple the chick so that it is unable to hatch. Semilethal genes prevent some but not all of the offspring from hatching. In addition, there are a number of genetically determined variations from the normal which cause death shortly after the chick hatches or seriously hamper its ability to survive in competition with normal birds. These are also frequently termed semilethal or sublethal traits, although, strictly speaking, the term lethal applies only to embryonic effects.

All the known, completely lethal genes are recessive. Dominant lethal mutations undoubtedly occur; but a dominant lethal mutation immediately eliminates the individual in which it appears and is therefore never transmitted. Autosomal recessive lethal genes which produce no morphological changes in heterozygous condition can be inherited for generations without revealing themselves until two individuals carrying the same lethal gene are mated. In such a mating, the chance expectation is that one-fourth of the offspring will inherit the normal allele from both parents and will therefore not be carriers of the lethal; one-half the progeny will inherit the lethal gene from one parent and the normal allele from the other parent and will appear normal but will be carriers of the lethal; and one-quarter of the offspring will inherit the lethal gene from both parents and will therefore die. However, unless attention is paid to the causes of embryo mortality and records of individual matings are kept, a recessive lethal gene may become widely dispersed in the population before a pronounced drop in hatchability occurs.

The presence of a lethal gene can often be detected by an unusually high embryonic mortality in a given mating. If the eggs are candled at regular intervals during incubation, the exact period of exceptionally high mortality can often be established, as for example at 4 days, or from 18 to 21 days. Examination of the dead embryos may reveal the nature of the lethal gene's effects. Such a procedure has led to the discovery of a number of lethal and semilethal genes in poultry.

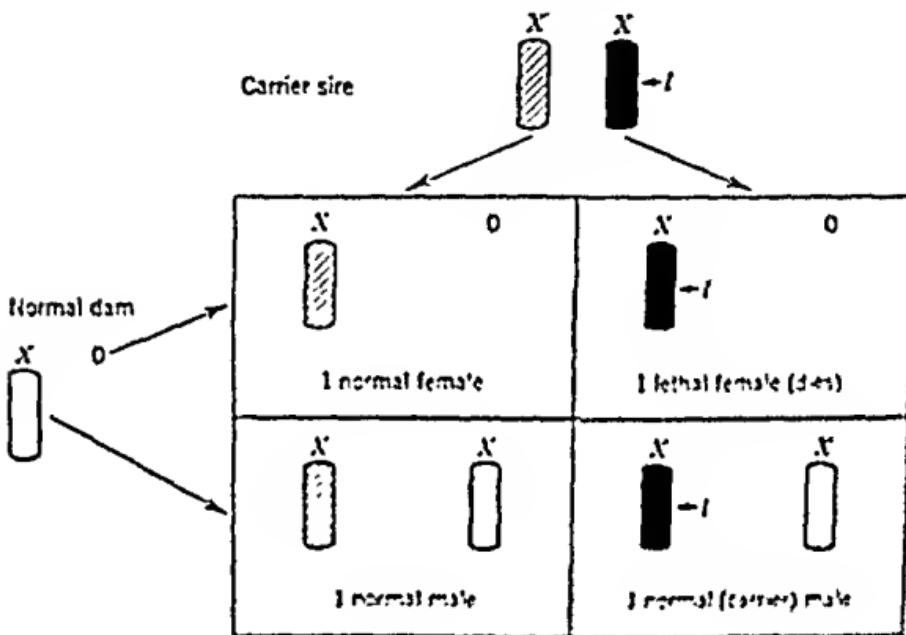
Sometimes a recessive lethal gene may have a slight semilethal effect in heterozygous condition; that is, certain individuals may succumb to a single dose of the gene despite the presence of a normal allele. Unfavorable environmental conditions or the general genetic background may be factors in such excessive suscep-

tibility of heterozygous embryos. Other lethal genes may have another type of effect in the heterozygote. One dose of the gene may produce morphological changes that are not fatal, and two doses may exaggerate the altered morphology to a fatal degree. This is true of the Creeper mutation which will be discussed below.

Recessive sex-linked lethal genes form a special class of lethals. In single dosage a sex-linked lethal has no effect on the male bird, as the second sex chromosome of the male carries a normal allele which prevents the expression of the lethal. But in the female embryo, a recessive lethal gene carried on the single sex chromosome acts like a dominant and is fully expressed. Therefore, the presence of sex-linked lethals can easily be detected by abnormal sex ratios. If the sire is heterozygous for a sex-linked lethal gene, all his sons, half of whom will inherit that lethal, will be normal, because they all derive a normal allele from the dam. Since the female bird inherits her one sex chromosome from the sire, half of the carrier sire's daughters inherit the lethal allele and die. The sex ratio of the progeny, then, is two males to one female instead of approximately one to one. Figure 52 illustrates the mode of inheritance of recessive sex-linked lethal genes.

In 1924 Dunn suggested that there might be numerous lethal and semilethal factors among poultry. At that time he had evidence of only one lethal gene in chickens. Since then eighteen more lethals have been demonstrated in chickens, and three have been found in turkeys.

is for half the progeny of the backcross to be white and half colored. In carrying out such a breeding program, Dunn obtained from backcross matings 51 colored and 29 white offspring instead of the expected 1:1 ratio. Some further matings produced



present. It was clear that this gene was closely or completely linked with the gene for white, as the white offspring were being selectively killed. Since the embryonic mortality was so high and since the lethal-bearing stock was also subject to high chick mortality, Dunn suggested that a single dose of this lethal gene might also decrease viability, that is, function as a semilethal. White Wyandottes have been in disrepute among many poultry breeders for poor hatchability, despite the fact that other breeders have had excellent results with them. Presence or absence of the recessive autosomal lethal gene found by Dunn may be the determining factor in these varying results. Evidence of the presence of this lethal gene in other strains of White Wyandotte fowl has been reported by Warren (1933). Hutt (1940) also found that inherited factors play a role in the poor reproductive capacity of this variety.

SEX-LINKED LETHAL IN WHITE LEGHORNS. The only other known lethal gene without visible morphological effects in poultry is a sex-linked lethal from inbred White Leghorns (Upp and Waters, 1935). Aberrant sex ratios were obtained from certain sires and some of their sons; females outnumbered the males two to one among 1200 dead embryos sexed, indicating a selective mortality of the female offspring of carrier males. The responsible gene was therefore adjudged to be a sex-linked recessive lethal.

CREEPER. A number of different types of micromelia (disproportionate dwarfism characterized by shortened extremities) occur in fowl; some are inherited; others are due to nutritional factors; and some are of unknown origin. Perhaps the most intensively studied genetic type is found in the Creeper fowl, which is also known as the Scotch Dumpie or Brevicrew (fig. 53). The same mutation or a very similar allele is present in the Japanese Bantam fowl (fig. 54).

The Creeper chicken is characterized by a marked shortening of the long bones of both wings and legs. The tibia is frequently bent and somewhat thickened; and the fibula, which in normal fowl (and other birds) consists of only a short splint attached at the upper end of the tibia, is highly differentiated to form a complete bone. The degree of shortening of the limbs varies con-

siderably; the majority of Creeper fowl are as vigorous as normal chickens, but extreme variants may be too crippled to survive. Abnormalities in differentiation of the cartilage and a deficiency in ossification (i.e., bone formation) of the cartilage within the shafts of the long bones during embryonic development are responsible for the Creeper condition. The specific histological pat-



FIG. 53. Creeper hen. (From Landauer and Dunn, 1930a.) Courtesy *Journal of Genetics*.



FIG. 54. Japanese Bantam male. (From Landauer, 1912a.) Courtesy *American Naturalist*.

tern places Creeper fowl in the class of chondrodystrophic or achondroplastic dwarfs.

Landauer and Dunn (1930a) also demonstrated that the Creeper gene in heterozygous condition has a slightly semilethal effect in the final stages of embryonic development. The frequencies of Creeper and normal offspring given above were based on classification of both hatched and dead-in-shell chicks. However, in matings involving all four strains of Creepers, the ratios consistently showed an excess of Creepers among the dead-in-shell, and a deficiency among the hatched chicks. It was concluded that the viability of heterozygous Creeper embryos is lower than that of normal ones. The more extreme Creeper variants probably have less chance of hatching and are also less viable after hatching.

Since the majority of homozygous Creeper embryos die at the beginning of the fourth day of embryonic development, early mortality in *inter se* Creeper matings is usually more than 25 per cent of the total number of fertile eggs set (table 40). Some

TABLE 40

SUMMARY OF EMBRYONIC MORTALITY AND HATCHABILITY IN CREEPER MATINGS
(Adapted from Landauer and Dunn, 1930a)

Mating	Number of fertile eggs	Mortality in per cent			Per cent hatched	Per cent phoko- melia
		1-6 days	7-16 days	17-22 days		
Creeper × Normal	1136	6.9	4.6	19.6	68.9	0
Creeper × Creeper (same line)	1444	28.5	4.4	26.7	40.4	1.6
Creeper × Creeper (outercross-differ- ent lines)	1714	23.3	3.6	16.6	56.6	2.0

homozygous Creeper embryos can be recognized by retarded growth and differentiation at 36 hours of incubation (Landauer, 1932a). Head and limbs are most severely affected (fig. 55). A few homozygotes survive this critical growth retardation and develop into phocomelic chicks which are unable to hatch. As fig. 56 indicates, the long bones in phocomelia (a more extreme form of micromelia) are greatly shortened, and the humerus and femur may be altogether missing except for minor rudiments. In addition, the head and eyes may be reduced in size or de-

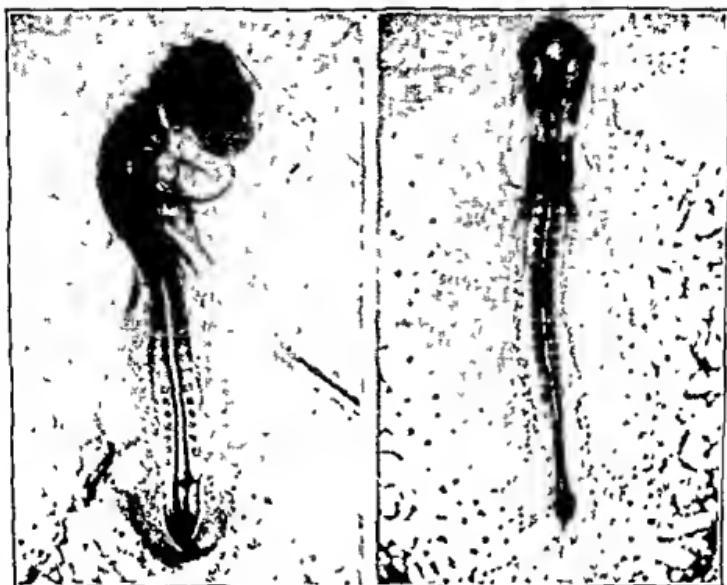


FIG. 55. Normal embryo after 48 hours of incubation (left) and homozygous Creeper embryo of the same age (right). (From Landauer, 1932a.)
Courtesy Journal of Genetics.



FIG. 56. Phokomelia: homozygous Creeper embryo after 19 days of incubation. (From Landauer, 1933.) Courtesy Zeitschrift für mikroskopische anatomische Forschung.

formed (Landauer, 1933). In crosses between the different geographic strains of Creepers, Landauer and Dunn (1930a) obtained a slightly higher percentage of survival beyond 4 days (see table 40) and a higher frequency of phokomelie embryos than were found in matings within the various strains. This greater survival of homozygous Creepers in outcross matings was attributed to hybrid vigor. Even more phokomelic embryos were obtained from matings between the Creeper offspring from outcrosses of Creeper to White Leghorn or Rhode Island Red stocks.

Crosses between Japanese Bantam and Creeper fowls by artificial insemination indicated that the mutations responsible for shortened extremities in these two varieties are either identical or are alleles with similar effects (Landauer, 1942a). It was observed, however, that in *inter se* Japanese Bantam matings there was no preferential embryonic mortality of individuals that were heterozygous for the lethal gene; that is, a single dose of the Creeper gene had no semilethal effects. Furthermore, in both *inter se* Japanese Bantam matings and crosses to ordinary Creepers, a higher percentage of homozygotes survived the fourth day and developed into phokomelic embryos (30 per cent or more as compared with 3 to 10 per cent). Landauer suggested that an incompletely dominant genetic modifier closely linked with the Creeper gene probably lessened to some extent the latter's deleterious effects in the Japanese Bantam breed.

CORNISH LETHAL. A second hereditary type of mieromelia was reported in Dark Cornish fowl by Landauer (1935). The standard of perfection in the Cornish breed demands short or moderately short shanks; the typical features are a broad head and body, legs short and set far apart, long bones of wings and legs strong, straight, and short (fig. 57). As in the Creeper fowl, the fibula is a well-developed bone. Bending of the tibia does not occur. Breeding tests with *inter se* Cornish matings, outcrosses to Leghorns and other varieties, and *inter se* matings of the hybrid



FIG. 57. Cornish male.
(From Landauer, 1935.)
Courtesy *Journal of Genetics*.

offspring led to the conclusion that several factors for short limbs are present. One of these is lethal in homozygous condition. Matings between short-legged carriers of this lethal produced 25 per cent of mieromelic embryos; many of them were alive at the end of incubation but unable to hatch. These embryos had extremely short limbs, short broad heads, and bulging eyes; in

figure 58 such an embryo is seen to be very similar to the phocomelic homozygous Creeper embryos though its appearance is somewhat less extreme. The Cornish lethal is therefore a single autosomal recessive gene with dominant morphological effects.

Reciprocal crosses between Creeper and Cornish stocks gave hatchabilities of more than 80 per cent and no extremely deformed embryos; thus it was proved that, despite their similarities, the Creeper and Cornish lethals are two entirely different and distinct mutations.

SNORT UPPER BEAK. Landauer (1911a) reported the discovery in a stock from Houdan cross-



FIG. 58. Micromelia in an embryo homozygous for the Cornish lethal. (From Landauer, 1935.) Courtesy *Journal of Genetics*.

bred ancestry of a recessive autosomal lethal gene which caused not only a shortening of the long bones but also a variable reduction in length of the upper beak (fig. 59). This lethal might have been derived from ancestors of the Houdan fowl, which is bred for short legs. Both the Creeper and Cornish types are also bred for short legs at the expense of propagating a lethal gene.

no correlation between the degree of reduction of the beak and of the long bones. As in Creepers and Cornish fowl, the long bones of the legs are affected more severely than those of the wings, and the upper bones of both wings and legs are shortened

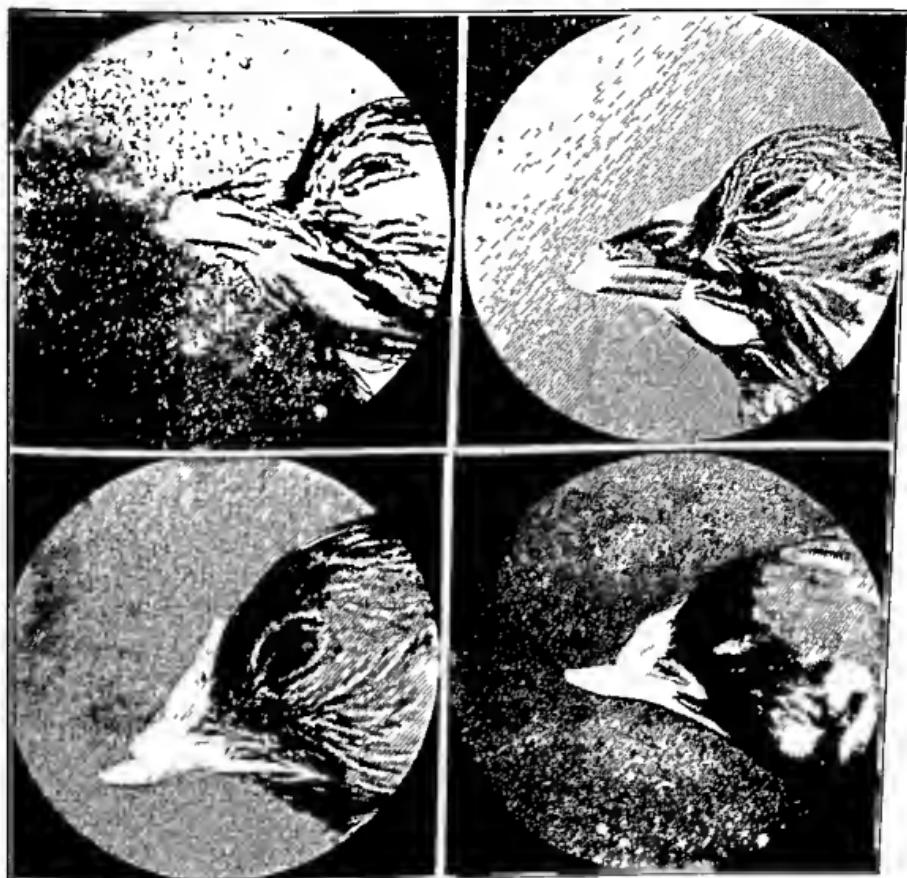


FIG. 59. Various degrees of beak reduction caused by the short-upper-beak lethal. (From Landauer, 1941a.) Courtesy *Genetics*.

less than the more distal bones. In the 13 per cent of afflicted embryos that succeeded in hatching, low viability and low fertility were reported. A gradual lengthening of the upper beak to approach the normal condition was observed in some survivors.

By selection of the least extreme variants as breeders each year, modifying factors have been accumulated in the short-upper-beak stock until both lethal and morphological effects have been all but eliminated (Landauer, 1946). It seems probable that

TABLE 41

MORTALITY DISTRIBUTION AND FREQUENCY OF SHORT-UPPER-BEAK MUTANT
 (Adapted from Landauer, 1941a)

Mating	Number of fertile eggs	Embryo mortality in per cent				Per cent hatched	
		1-6 days		7-18 days		Short upper beak	Total
		1-6 days	7-18 days	Short upper beak	Total		
Normal ♀♀ × heterozygous ♂	675	5.0	4.6	14.5	...	75.9
Normal ♀♀ × homozygous ♂	73	9.6	5.6	6.8	...	78.1
Heterozygous <i>inter se</i>	742	7.1	5.0	19.4	33.3	3.0	54.6
Heterozygous ♀♀ × homozygous ♂	204	6.5	8.5	35.0	46.3	5.4	39.5

some modifiers were already present in the original material in which the lethal was found; they would explain the great differences in expression and hatchability observed in different matings. Landauer at first reported the gene to be a semilethal but later concluded that, in the absence of modifiers, it would probably be completely lethal.

CHONDRODYSTROPHY (LAMOREUX). Lamoreux (1942) described a fourth distinct type of mieromelin which he termed hereditary chondrodystrophy. The mutation, which occurred among inbred White Leghorns, appeared to be a simple autosomal recessive lethal with a wide range in expression. Out of 1713 progeny from matings between carriers, 22.5 per cent were classified as chondrodystrophic. Some matings produced extreme variants exclusively; others produced a so-called "modified" expression, some individuals being almost normal in appearance. Some modified chicks were able to hatch. Lamoreux suggested that it was possible that heterozygous individuals occasionally developed a

milder form of chondrodystrophy, or that modifying genes sometimes altered the severe effects in homozygotes.

Typically, affected embryos of the extreme type had short bent tibiae and tarsometatarsal bones, a less extreme curvature and reduction of the long bones in the wings, a curved upper beak, and short lower mandible (i.e., parrot beak). Figure 60, reproduced from Lamoreux's report, shows the essential features of the mutant. There was no indication of a general retardation in growth, and many chondrodystrophic embryos were alive but unable to hatch at the end of incubation. Histological examinations have revealed abnormalities in cartilage and bone formation which seem to justify the classification of this abnormality as chondrodystrophy (achondroplasia). Both gross and microscopic anatomy appear to be identical with those of sporadic or accidental chondrodystrophy.

MICROMELIA. A fifth genetically determined type of micromelia appeared among the descendants of a cross between White Leghorns and Bantams (Asmundson, 1942a). On the basis of the breeding data (716 normal to 47 mieromelic) Asmundson suggested that two pairs of autosomal recessive genes were involved. Neither of the gene pairs, apparently, had any effect in single dosage. The micromelic embryos appeared superficially similar to the Cornish lethal mieromelics, but outerosses to carriers of the Cornish lethal produced all normal progeny; thus it was proved that the two types of micromelia are independent. Asmundson's micromelia is characterized by very short thick legs, a short deformed lower beak, parrot upper beak, and usually a vaulted skull (fig. 61). The long bones are much wider, or thicker, than normal. The proximal long bones are shortened relatively more than the distal



FIG. 60. Extreme chondrodystrophy in a day-old help-out chick. (From Lamoreux, 1942.) Courtesy *Journal of Heredity*.

TABLE 41

MORTALITY DISTRIBUTION AND FREQUENCY OF SHORT-UPPER-BEAK MUTANT
(Adapted from Landauer, 1941a)

Mating	Number of fertile eggs	Embryo mortality in per cent				Per cent hatched	
		1-6 days		19-22 days		Short upper beak	Total
		7-18 days		Short upper beak	Total		
Normal ♀♀ × heterozygous ♂	675	5.0	4.6	14.5	...	75.9
Normal ♀♀ × homozygous ♂	73	9.6	5.6	6.8	...	78.1
Heterozygous <i>inter se</i>	742	7.1	5.0	19.4	33.3	3.0	54.6
Heterozygous ♀ ♀ × homozygous ♂	294	6.5	8.5	35.0	46.3	5.4	39.5

some modifiers were already present in the original material in which the lethal was found; they would explain the great differences in expression and hatchability observed in different matings. Landauer at first reported the gene to be a semilethal but later concluded that, in the absence of modifiers, it would probably be completely lethal.

CHONDRODYSTROPHY (LAMOREUX). Lamoreux (1942) described a fourth distinct type of micromelanin which he termed hereditary chondrodystrophy. The mutation, which occurred among inbred White Leghorns, appeared to be a simple autosomal recessive lethal with a wide range in expression. Out of 1713 progeny from matings between carriers, 22.5 per cent were classified as chondrodystrophic. Some matings produced extreme variants exclusively; others produced a so-called "modified" expression, some individuals being almost normal in appearance. Some modified chicks were able to hatch. Lamoreux suggested that it was possible that heterozygous individuals occasionally developed a

banded in the spring of 1946. Of these, one male which was brooded and raised on the range in competition with the general flocks has survived and is still alive at the age of a year and some months. This cockerel is considerably smaller than normal for the Rhode Island Red breed (fig. 63).

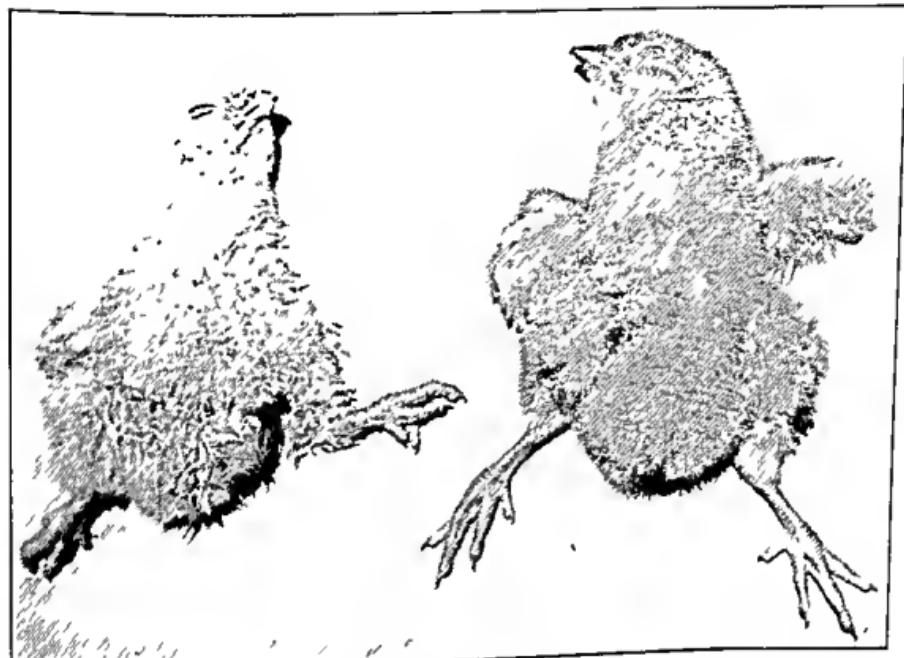


FIG. 62. Micromelia (left) in a Rhode Island Red chick at time of hatching. A normal chick is shown at the right. (From Hays, 1944.) Courtesy *American Naturalist*.

In these six micromelias of genetic origin, the structure of the head is also affected. Such simultaneous effects on head and limbs are not peculiar to hereditary forms of micromelia only but occur together also in the various nutritional types, in experimentally induced micromelia, and in the sporadic or accidental types of unknown origins.

Chondrodystrophy, or achondroplasia, is a form of micromelia which is characterized, according to the classical description, by clear-cut irregularities in the arrangement of the columnar zone of the epiphyseal cartilage. Furthermore, the abnormalities in bone formation are limited to endosteal bone (the spongy internal bone), whereas periosteal bone (the outer layer of the shaft of

ones, a condition which is the reverse of the usual situation in most forms of mieromelia. The gene combination is lethal at the end of incubation.

"CHONDRODYSTROPHY" (HAYS). The sixth lethal micromelia appeared in Rhode Island Reds (Hays, 1944) among the progeny of an inbred mating. Two full-sisters and five half-sisters mated

to their brother produced a total of 172 normal and 54 micromelic offspring. Hays concluded that the abnormality was dependent on an autosomal recessive gene which was lethal when homozygous. The postembryonic mortality among the normal progeny of carrier females was considerably higher than it was among the offspring of noncarrier females; thus a delayed lethal effect of the gene was indicated in heterozygous condition as well, although no morphological effects were apparent.



FIG. 61. Micromelia, the lethal effect of two pairs of recessive genes. (From Asmundson, 1942a.) Courtesy *Journal of Heredity*.

This form of micromelia is somewhat less extreme than some of the other known forms. The long bones of the limbs are shortened, with the humerus, ulna, and tarsometatarsus thickened. The structure of the femur is abnormal, according to Hays; the bone appears to be crumbling, with the fibrous covering (periosteum) incomplete and indistinct. All the leg bones are straight and in normal position. The head is wide and flat, and the beak is blunt. Both upper and lower mandibles are shortened, but normally proportioned, with no parrot-beak curvature. The size of the fully developed embryo appears normal (fig. 62). As yet, histological justification for terming this mutation "chondrodystrophy" is lacking.

Some of the micromelic chicks hatch. Hays reported that none of these lived longer than a week. However, at the Storrs Agricultural Experiment Station a few micromelic specimens were

ing possible relationships between the vitamin and the lethal gene. Certainly it is known that nonhereditary stickiness does occur.

Byerly and Jull reported that mating tests between carrier birds gave 545 normal to 169 "sticky" progeny, which is close enough to the expected 3:1 ratio (535.5 to 178.5) to indicate that a single autosomal recessive mutation causes the sticky condition.



FIG. 64. Normal and talpid embryos of 17 days' incubation. (Photograph supplied by R. K. Cole; talpid embryo previously used by Cole, 1942, in the *Journal of Heredity*.)

The lethal appeared to have been derived from a Barred Plymouth Rock strain. Hatchability was so low in matings between carriers (36.6 per cent) that the investigators suggested either that the sticky gene was semilethal in heterozygous condition or that other lethal genes were also present in the stock.

TALPID. Another gene producing simultaneous changes in beak and limb morphology is the talpid lethal, which was uncovered as a result of inbreeding among White Leghorn fowl and described by Cole (1942). Figure 64 shows the principal feature of the talpid embryo, an extreme polydactylism (extra toes and wing digits). There may be as many as six or seven (in extreme

the long bones) forms normally. Not all the hereditary micro-melias have had detailed histological examinations. Only two have been proved by such studies to belong to the chondrodystrophic group: the Creeper and Lamoreux's chondrodystrophy.

STICKINESS. There are certain other mutations with simultaneous effects both on the limbs and on the head or beak. One of these, chronologically the third lethal to be discovered in chick-



FIG. 63. Rhode Island Red micromelic cockerel. *Photograph from Storrs Agr. Expt. Sta.*

ens, was described by Byerly and Jull in 1932. At the end of the incubation period the fully developed embryos are immersed in abnormally viscous amniotic and allantoic fluids which they have failed to swallow or absorb. This results in a unique stickiness for which the lethal is named. In addition, there is more or less edema of the body, which is smaller than normal, the beaks are curved downward, the tibiae frequently are bent, and the abdomens are distended with unabsorbed yolk. Calcium does not appear to be withdrawn from the shell during development, as is normal, and the bones remain soft and rubbery (uncalcified). These embryos are completely unable to hatch. Ogorodniy and Penionschkevitsch (1939) suggest that stickiness may result from the disturbed water and protein metabolism ensuing from riboflavin deficiency. No information is available, however, regard-

and legs are shortened and frequently curved, the tibia being most severely affected. The majority of diplopod embryos also have short upper beaks. Approximately 2 per cent were able to hatch but did not survive. Figure 65 shows a diplopod embryo with typical development of the legs. The wings in this individual, however, show a more extreme duplication than is usual.



FIG. 65. Diplopod embryo showing supernumerary wing and leg structures. The development of the legs is typical; that of the wings is more extreme than usual. (From Taylor and Gunns, 1947.) Courtesy *Journal of Heredity*.

Since several matings between known carriers produced close to the expected ratio of diplopod offspring (that is, one-fourth of the total progeny), Taylor and Gunns deduced that a single autosomal recessive gene was responsible for the abnormality. Out of 1531 offspring, 348 (22.7 per cent) were diplopods. However, among the descendants of an outcross to unrelated White Leghorn stock, there appeared a number of so-called atypical carriers which produced unexpected deficiencies of diplopods among their progeny by carrier males. Some of their phenotypi-

cases, nine or ten) digits per limb. These are usually of approximately equal length, and webbed or fused. The extra wing digits form a structure which, according to Cole, frequently resembles a hand. In addition, the upper bones of the wings and legs are considerably shortened; facial and beak development are retarded and abnormal; the upper and lower beaks are separated; the viscera are ectopic (protruding and exposed, rather than covered by the body walls); the spinal column is shortened; blisters occur under the skin of the thigh, head, and neck; feather formation is retarded; and development in general is slower than normal.

Cole found abnormalities of limb bud development to be visible by the fourth day of incubation, although deficient numbers of talpid embryos from pedigree matings suggested that the gene might occasionally be lethal even before then. The majority of talpid embryos died before the end of the tenth day; the maximum survival reported by Cole was 17 days. If it is assumed that an autosomal recessive gene produces the talpid syndrome, the observed low frequency of 16.6 per cent of abnormal embryos from all matings between carriers may be due to inability to classify many early dead embryos. From matings in which at least 95 per cent of the total progeny was classified, 98 out of 415 embryos were talpids, that is, 23.6 per cent. This is in satisfactory agreement with the expected 25 per cent for a single autosomal recessive gene.

DIPLOPODIA. A second type of lethal polydactyly, diplopodia, also occurring in White Leghorns, was described by Taylor and Gunns (1947). In over 75 per cent of the diplopod embryos there are six toes per leg in groups of three toes each, the extra group apparently having been substituted for the hallux, or first toe. The supernumerary digits are variable in size and structure. Twenty per cent of the diplopods have more or less than three toes in the extra complement, and in a few individuals the hallux plus an extra group are present. Some of the embryos are asymmetrical with respect to polydactylyism; in five-sixths of these, the right leg has more toes than the left. A striking duplication of the metatarsal bones with which the toes articulate is also characteristic. Almost all diplopod specimens have duplications of the corresponding wing structures. The long bones of both wings

teenth day, growth slows markedly, and, after the sixteenth day, little if any further growth occurs. Dwarf embryos at 21 days are, therefore, much lighter in weight than normal embryos. An abnormal amount of amniotic fluid may also be present. Rosenberg (1947) has reported that the cells of the skeletal muscles of the crooked-neck dwarf undergo degeneration and diminish in volume. By the nineteenth day, degeneration is at an advanced stage, and the muscles are stringy and nonfunctional.

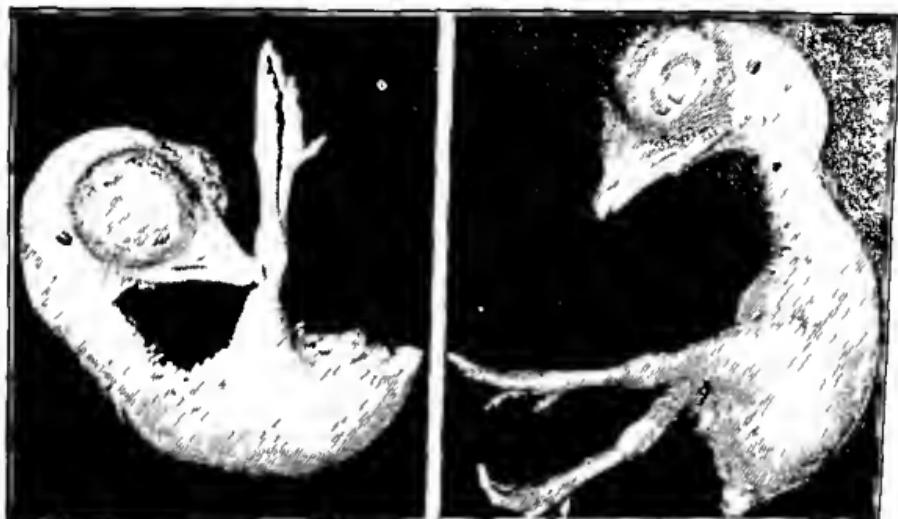


FIG. 67. Lethal wingless embryos of 11 days' incubation. Embryo at left is completely wingless. Embryo at right has small wing rudiment.

Photographs supplied by Edgar Zwilling of the Storrs Agr. Expt. Sta.

WINGLESS. Another autosomal recessive lethal gene causes a wingless condition in Single Comb White Leghorns (Waters and Bywaters, 1943). This single gene has extremely complex multiple effects, which are already apparent by the fourth day of development. The wings are completely absent or, occasionally, exist as a very slight rudiment of the humerus; the legs are present, but are usually abnormal, with irregularities in the joints, with rudimentary, absent, duplicated, fused, or misplaced toes, and with rudimentary scales. The lungs and air sacs are absent, and the definitive kidneys are completely missing or represented by fragmentary patches of tissue. The down is clubbed. Wingless embryos may survive the incubation period, but they never hatch. Figure 67 shows two wingless embryos of approximately

cally normal offspring proved in turn also to be atypical carriers. The workers suggested that the outcross might have introduced certain modifying genes which functioned as suppressors of the lethal.

CROOKED-NECK DWARF. Asmundson (1945) described the crooked-neck dwarf, a lethal abnormality which occurred in the New Hampshire breed. From matings in families that segre-

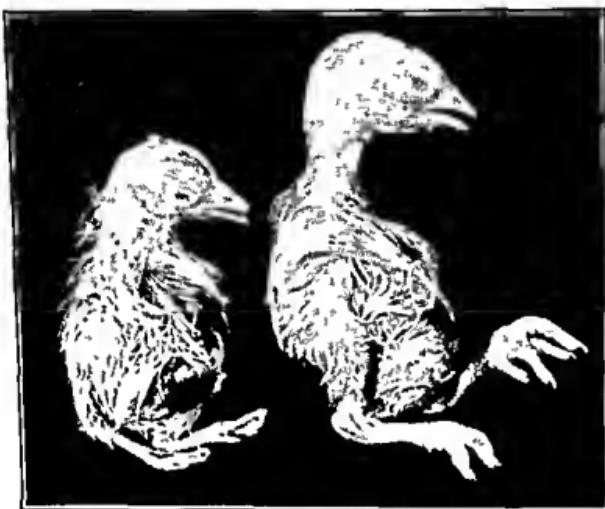


FIG. 66. Crooked-neck-dwarf (left) and normal embryo (right). (From Asmundson, 1945.) Courtesy *Journal of Heredity*.

gated for the lethal, Asmundson obtained a total of 297 normal and 103 dwarf embryos, a good 3:1 ratio indicating a single autosomal recessive gene as the causative agent. The gene has no effect in heterozygous condition. Affected embryos die on the twentieth or twenty-first day or are found alive but unable to hatch on the twenty-second day.

A crooked-neck-dwarf embryo is pictured in figure 66. Both skeleton and musculature are altered by this gene. Typically, the upper beak is somewhat shortened, the neck is crooked, the keel and lateral processes of the sternum (breastbone) are absent, and other parts of the skeleton (long bones) may be slightly reduced. The breast and leg muscles are extremely reduced, and living, full-term embryos cannot flex their limbs. At 11 to 13 days, dwarf embryos are edematous; beginning with the thir-

day of development, and the lethal is effective at time of hatching, as the chicks usually cannot pip the shell. One out of 58 abnormal chicks hatched but did not survive more than a few days according to Asmundson. An almost perfect 3:1 ratio (173 normal to 58 abnormal) indicated that the deformity is caused by a single autosomal recessive gene which has no effects in heterozygous condition.

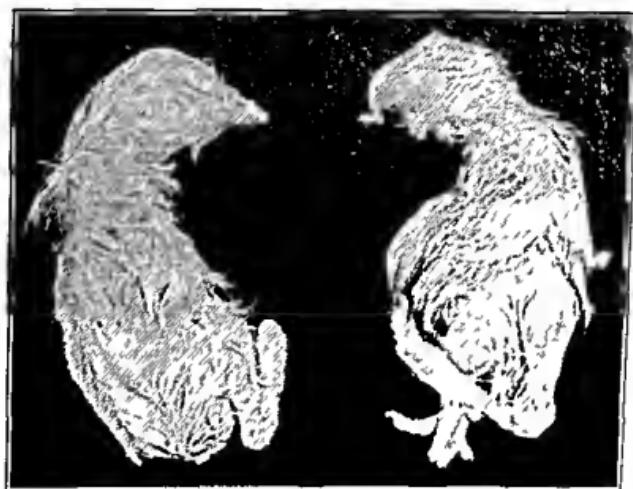


FIG. 69. Lethal deformity of the mandibles in 21-day embryo at right. Normal embryo at left. (From Marble, Harper, and Hammers, 1944.)
Courtesy Poultry Science.

DEFORMED MANDIBLES. Marble, Harper, and Hammers (1944) described another single autosomal recessive lethal mutation which reduces the beak in White Leghorns. Out of 512 progeny, 134 were deformed. The effects of this gene are more severe than those of Asmundson's amaxilla. The upper beak is considerably reduced and pointed or curved upward; only a vestige of the lower beak remains (fig. 69). Cerebral hernia is always present, and other parts of the face, particularly the eyes, are frequently deformed. Abnormalities in development are apparent at 8 days of incubation, although the lethal effects are not evident until the time for hatching, a feat which the deformed embryos cannot accomplish.

SHOOT LOWER MANDIBLE. A third recessive mutation that alters beak development also occurred in White Leghorn fowl and was

11 days incubation. One of these is completely wingless; the other has a small vestigial wing stump.

AMAXILLA. Many of the lethals already discussed affect the head and beak in addition to other parts of the body. There are

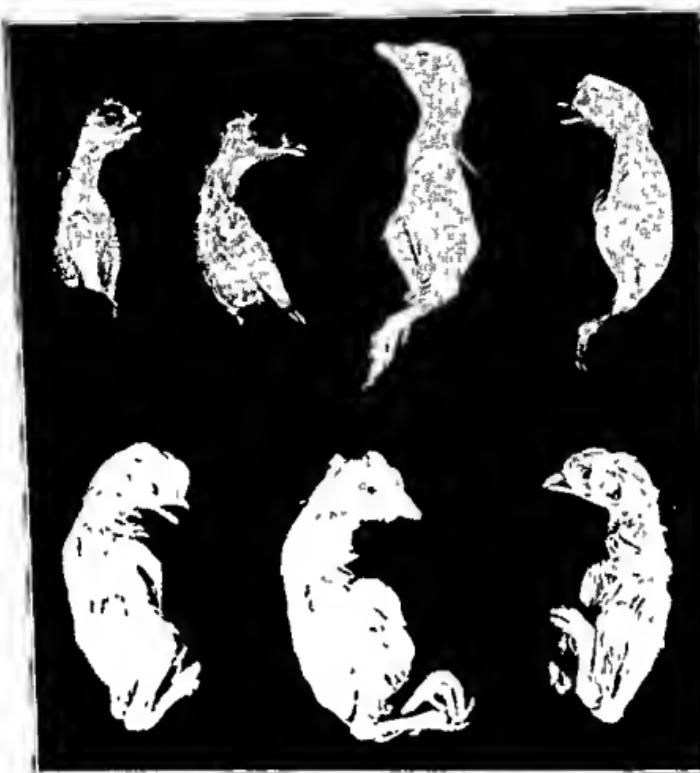


FIG. 68. Amaxilla. Upper row, in order from left to right, shows abnormal and normal 15-day embryos, normal and abnormal 18-day embryos. Below, two abnormal embryos (left and right) and a normal embryo (center) of 21 days' incubation. (From Asmundson, 1936.) Courtesy *Journal of Heredity*.

also a few the primary effects of which are on the beak. Asmundson (1936) discovered the first of these, amaxilla, among the progeny of matings of a White Leghorn male to his dam and several sisters. The maxillae are absent or reduced, and the upper beak is frequently bent to one side (fig. 68). The affected embryos may be otherwise completely normal, although the premaxillary and nasal bones and the eyes are sometimes also smaller than normal. The deformity is recognizable after the twelfth

homozygotes failed to hatch. He found no indication of the stage at which death usually occurred.

NAKED. The second semilethal gene affecting the plumage in chickens, naked, is completely recessive and sex-linked (Hutt and Sturkie, 1938). Half of the affected embryos die between the



FIG. 70. Defective plumage caused by the flightless gene in heterozygous dosage. (From Warren, 1937.) *Courtesy Journal of Heredity*

reported by McGibbon (1946). The lower beak usually is not more than half as long as the upper beak, and the latter is frequently curved downward, giving a parrot-beak appearance. Buckling in the mid-region of the mandible, accompanied by a rolling under of the tongue, may also occur. Irregularities are visible by 12 days of development, and 50 per cent of the afflicted chicks fail to hatch; the gene is therefore a semilethal. Only a few of the hatched chicks survive. Matings between carrier individuals and between carrier and affected fowl gave ratios indicating that a single recessive autosomal gene is responsible for short lower mandible. However, in one mating between a pair of short-lower-mandible birds, 37 abnormal and 2 normal progeny resulted. McGibbon suggested that the penetrance or expressivity of the gene may be variable.

FLIGHTLESS. Of two semilethal mutations the primary morphological effects of which are on the plumage, the first, flightless, was described by Warren (1932, 1937). Defects in the shafts of the flight feathers and of the larger feathers in the tail and other parts of the body cause the feathers to break off near the base at the slightest pressure. Flightless chicks appear normal at hatching, but by the age of 4 weeks, the abnormality of the feathers is apparent (fig. 70).

Breeding experiments suggested that the gene involved is an autosomal dominant, which is semilethal when homozygous. Since 31 crosses between flightless and normal fowl each produced some normal offspring and since the total progeny from such crosses comprised about equal numbers of normal and flightless individuals, it was assumed that all the flightless birds tested were heterozygotes. In matings between flightless individuals with a hatchability of 74 per cent, Warren obtained 116 flightless, 51 normal, and 16 featherless progeny. The featherless chicks had normal down, but the failure of the pin feathers to develop after their first appearance left the skin almost bare (fig. 71). Retardation of general growth as well as of feather development was apparent in a few weeks. The beaks and toe nails also became deformed, as a result of brittleness and breaking. The few featherless birds that were reared never attained sexual maturity. Warren concluded that the featherless birds were probably homozygous for the flightless gene and that the majority of

unerupted feather follicles. By transplanting sections of normal skin to naked hosts the authors demonstrated that the mutation primarily affects the feather follicles; normal feathers developed in the region of the transplant.

The same or a very similar mutation may have been observed earlier by Serebrovsky and Petroff (1929), who found three unhatched (two dead) chicks with very little down and poorly developed scales among the offspring of inbred matings. These investigators suggested multiple recessive semilethal genes as a possible cause, but the data were far too limited for adequate conclusions.

MICROPHTHALMIA. Jeffrey (1941) reported the discovery in Barred Plymouth Rocks of a semilethal mutation that causes a reduction in size of the eyeball (microphthalmia) and a thickening or doubling of part or all of the comb. The eyes have only half the normal diameter. A large proportion of the affected individuals fail to hatch, and the chicks that do hatch are blind and unable to survive. A ratio of 196 normal to 60 microphthalmic offspring from matings between carriers indicated the activity of a single autosomal recessive gene. Further breeding tests gave ratios corroborating this interpretation.

Gruenwald (1944) made histological studies of this abnormality and found very early in development that cysts and folds appear in the retina and to a lesser extent in the iris and ciliary bodies. These cysts and folds later degenerate. By 5 or 6 days, the reduction in size of the eyeball is grossly visible.

OTHER DELETERIOUS GENES. In addition to these nineteen established lethal and semilethal mutations in chickens, several other genes have been reported to have lethal effects, but substantial evidence is lacking. Furthermore there are a number of mutations that have little or no effect on hatchability but cause the death of the affected chicks shortly after hatching, or cripple them to such an extent that they cannot survive under ordinary conditions of rearing. These latter mutations are frequently referred to as semilethals, sublethals, or delayed lethals, and a few of them are included in Lerner's (1944) checklist of lethal characters in farm animals.

We have already referred to one of the inconclusive claims for lethal genes, that of Serebrovsky and Petroff (1929) concerning



FIG. 71. Featherless chick (probably homozygous for the flightless gene) from mating between flightless parents. (From Warren, 1937.) Courtesy *Journal of Heredity*.



FIG. 72. Naked chick. Photograph supplied by F. B. Hutt, Cornell University.

the first week, the trembling gradually diminished in intensity, disappearing entirely after about 8 weeks but leaving the chicks retarded and stunted. The exact mode of inheritance of congenital tremor is undetermined. A ratio of 408 normal to 39 trembling chicks (10.5:1) was obtained from matings that produced abnormal offspring. Hutt and Child suggested that two pairs of genes may be responsible, but they considered it more likely that a single gene pair modified by other genes to a low penetrance causes the condition of tremor.

A high postnatal mortality was also reported among White Leghorn chicks that were blind as a result of the action of a single autosomal recessive gene (Hutt, 1935). The blindness is associated, according to Hutt, with a bulging of one or both eyes and frequently with atrophy of the optic nerve. No detailed information regarding this condition has been published.

A dominant autosomal gene causing various degrees of nakedness as a result of the absence of feather follicles was reported by Sturkie (1942) in Rhode Island Red fowl. Hatchability is not affected, but more than half the naked chicks die within 2 weeks after hatching. Heterozygous and homozygous effects of the gene appear to be identical.

Another so-called sublethal gene, which causes stringy down and eliminates all affected chicks before they are 2 weeks old, was reported by Kessel (1945). Again, hatchability *per se* appears to be unaltered.

Bohren and Waters (Bohren, personal communication) have evidence that a single recessive gene may be responsible for a slipped-tendon condition, which they call "congenital perosis," in newly hatched chicks.

A different type of lethal effect has been suggested by Kondireff (1925). Malformations of the legs that appeared in the F_2 generation of a cross between Bantams and Minorcas were ascribed to disharmonious gene segregations resulting from the crossing of a large with a small breed. The data from this study are not conclusive. However, such an hypothesized incompatible gene combination could certainly have lethal effects. A process of this type may be effective in the morphological distortions and high embryonic mortality characteristic of intergeneric crosses. (See Landauer, 1941c, for references.)

a fatal, downless condition which appeared in three inbred embryos. Likewise Gericke (1934) had insufficient evidence to establish his contention that a lethal mutation had occurred in a stock of Black Australorps in which certain hens turned white. Czaja (1939) suggested that pelvic and spinal abnormalities in a stock of Polish Greenfoot fowl were due to several recessive genes which were semilethal at relatively advanced ages. The condition described by Czaja may have been caused in part by hereditary tendencies, but the data were inadequate, and, in any event, the designation semilethal appears to be unwarranted.

One of the inherited conditions that are fatal just after hatching is congenital loco, a mutation described by Knowlton (1929) in Barred Plymouth Rocks. Congenital loco is characterized by a lack of control of the neck muscles; the head is bent over backwards, and the chicks fall frequently. The same type of abnormality has also been observed in White Leghorn, Rhode Island Red, White Plymouth Rock, White Wyandotte, and Ancona fowls. In Knowlton's stock, matings between proved carriers produced 146 loco out of 607 progeny, that is, 24 per cent. A single autosomal recessive gene, which apparently does not affect hatchability, determines congenital loco.

Mayhew and Upp (1932) and Upp (1934a) accumulated data on the inheritance of a form of dwarfism which was first described by Landauer (1929). Dwarf chicks, which cannot always be accurately distinguished until 2 weeks after hatching, have short legs, wide heads, and parrot-like beaks, are subnormal in size and weight and difficult to raise. It was concluded from the breeding data that a single autosomal recessive gene probably was responsible, although there was a slight deficiency in the number of dwarf offspring to be expected from this type of inheritance. Upp suggested that possibly there was a selective mortality of dwarf progeny before they could be recognized. However, a true semilethal effect has not been adequately demonstrated.

Hutt and Child (1934) reported congenital tremor to have no preferential prenatal mortality, but it is fatal to two-thirds of the afflicted chicks within a week after hatching. Abnormal chicks tremble violently whenever they stand, as many as 17 vibrations per second having been recorded. In chicks surviving

SEX-LINKED ALBINISM. Hutt and Mueller (1942) described an imperfect albinism that occurred in purebred Bronze turkeys. All the albino offspring of carrier males were females and thus indicated a sex-linked recessive gene to be responsible. Pigment development is almost, but not completely, suppressed; the plumage color is a dingy white, with faint indications of the Bronze color pattern (fig. 73).

Dead-in-shell embryos are cream-colored. The albinos are blind; their eyes are pale blue-grey, with red pupils.

The albino gene apparently has effects other than those on pigment formation, since three-fourths of the albino embryos died between the twenty-fourth and twenty-eighth days of incubation. It seems likely that a preferential mortality of albinos is in effect at still earlier stages of development, as there is a deficiency of albinos among embryos older than 24 days (viz., 45 albino to 184 colored), according to the expected frequency for a single recessive gene (57.25:171.75).

SHORT SPINE. The short-spine mutation appeared among inbred Bourbon Red turkeys (Asmundson, 1942b). The necks and bodies of short-spine mutants (fig. 74) are shortened by a crowding together of the vertebrae. The legs and wings are completely normal, but the head is often abnormal, with reduction in the skull, eyes, and upper beak. Bone analyses revealed a decreased ash percentage of certain of the bones in the pectoral and pelvic girdles.

Breeding data suggest that a single autosomal recessive gene causes the short-spine condition. Of 27 short-spine embryos, none hatched. The gene is therefore assumed to be completely lethal.

SHORT. Asmundson (1944) also described a semilethal mutation in turkeys that produces a shortening of the long bones of



FIG. 74. Short spine lethal mutation in turkeys. (From Asmundson, 1942b.) Courtesy Society for Experimental Biology and Medicine.

Numerous other mutations seriously alter the viability of the hatched chick, and some affect embryonic survival and hatchability. Any genes that cause morphological abnormalities may be undesirable when the chicks are reared under competitive circumstances; these same genes frequently lower hatchability somewhat. Genes causing albinism, rumplessness, polydactylism of various sorts, frizzled plumage, crossed beaks, and the like may all fall into this category.

Turkeys

Only one lethal and two semilethal genes have thus far been recorded for turkeys. However, it is likely that lethals are just as numerous in turkeys as they are in chickens, and with increased pedigree breeding more lethals will undoubtedly be uncovered and described in the future.



FIG. 73. Lethal albinism in one-day-old turkey (left). Normal Bronze turkey poult at right. (From Hutt and Mueller, 1942.) Courtesy *Journal of Heredity*.

As Asmundson points out, the widespread preferential selection of shorter-limbed birds as breeders may result in actual selection for a lethal gene. The same thing has happened in Creeper, Cornish, Japanese Bantam, and possibly in Houdan chickens. Careful checks on the hatchability records and examination of the dead-in-shell chicks and poult should therefore be of great value in enabling poultry breeders to determine whether the type of selection being used may in the long run be detrimental to the flock.

SEX

Chickens

Extensive studies of the sex ratios of hatched chicks and dead-in-shell embryos have been made in an attempt to determine whether there is a preferential prenatal mortality of either sex. Significantly different sex ratios for hatched chicks have been observed in the various investigations. It is probable that the inconsistent findings may be traced to inherent differences in the genetic makeup of the various strains and breeds from which the data were obtained. For instance, Dudley and Hindhaugh (1939) observed the following sex ratios (which are expressed as the percentage of males) in their material: White Leghorns—55.2; White Wyandottes—49.6; and Rhode Island Reds—50.3. On the other hand, Hazel and Lamoreux (1946), using another strain of White Leghorns, found the sex ratio of 8355 chicks from 464 full-sib families to be 49.79. Furthermore they found the differences in sex ratio between families to be no greater than could be accounted for by chance distribution.

Jull (1931a) from a collection of all available data and from his own observations concluded that the normal sex ratio just after hatching is slightly under 50, that is, that female chicks exceed males in domestic chickens. Landauer and Landauer (1931) from an analysis of the literature also observed a significant deficiency of males among hatched chicks. The combined data for 67,993 chicks gave a sex ratio of 48.77, with a probable error of 0.13. They suggested that a preferential mortality of males prior to hatching might be the cause of the deficiency among hatched chicks. The higher metabolic rate of males was advanced as a possible explanation for greater mortality of the

the legs and wings (fig. 75). The upper bones of the limbs are altered less than the more distal ones; the tarsometatarsus shows the greatest decrease in length, and the tibia is frequently bent. The shortened bones are all thicker than normal, and this increase in width compensates for the decrease in length to such an extent that, except for the femur, there is no loss in bone weight. In addition, the neck vertebrae are shorter and wider than normal.



FIG. 75. Short and normal turkeys. *Photograph supplied by V. S. Asmundson, University of California.*

The syndrome of abnormalities appears to be very similar to mieromelia in chickens.

The short mutation occurred in the Bronze variety. Of 184 short-limbed embryos, only 25 hatched, and of these, 6 were raised. The segregation ratio of 590 normal to 184 short progeny indicated that a single autosomal recessive gene was involved. There was no effect on embryonic viability until time of hatching. Asmundson reported that careful comparisons between normal and carrier (heterozygous) individuals have revealed that the gene also has some dominant effects. Although the shank length of carrier turkeys is within the normal range, the heterozygotes, as a group, show a 7 per cent decrease in shank length from the normal. The short gene is therefore a recessive semi-lethal, with slight dominant morphological effects.

However, Asmundson (1941) found that, although 56.44 per cent of 326 sexable embryos dying before the fourteenth day were males, there was a preferential mortality of females during the last week of incubation (47.94 per cent males out of 1769 embryos). The sex ratio of over 5000 hatched chicks was also below 50. According to the total data available to Asmundson from the literature, 48.7 per cent of 35,513 dead embryos were males, whereas for 114,536 hatched chicks, the sex ratio was 49.35. These figures included the data on embryos reported by Crew, but not the data on hatched chicks which Crew obtained from the hatcheries. Asmundson concluded that, at least during the last week of incubation, more female than male embryos die. For earlier mortality, no data are sufficiently extensive to be conclusive.

In view of the contradictory findings it is perhaps impossible to come to any definite conclusion regarding the influence of sex on hatchability. In the first place, there is no adequate method of determining the primary sex ratio on a large scale. Secondly, few data on sex incidence among embryos dying at early stages of development are available. The indications at present are that a considerable excess of males die during the early part of the incubation period, whereas female mortality is greater during the later part. The sex ratio at time of hatching is generally below 50. Since it has been effectively demonstrated that strain and breed differences in sex ratio are significant, it is probable that the genetic makeup is responsible for variations in primary sex ratio and/or mortality differences between the sexes. The sex of the individual may influence, possibly via differences in metabolic rate, the susceptibility of the embryo to various environmental conditions that may affect hatchability. For example, Landauer (1943) found the malformation otocephaly to occur more frequently among females than among males, and Byerly and Jull (1935) noted that sporadic exoadrodystryphy affects more males than females.

Hays (1941b) has made the interesting observation, based on timed records for over 4000 Rhode Island Red chicks, that females predominate among chicks that hatch first in any given setting and males are in excess among those that emerge after the first 24 hours of the hatching period.

male sex. However, Byerly and Jull (1935) from their own and other data determined that there is a significant preferential prenatal mortality of females rather than of males. Among 24,853 dead embryos sexed, only 47.85 ± 0.21 per cent were males. Landauer (1943) also observed that females exceeded males in the dead embryos from crosses between Barred Plymouth Rock females and Rhode Island Red males.

Crew (1938) collected sex-ratio records from various hatcheries. Out of 2,216,051 chicks sexed by the Japanese method of cloacal palpation, 51.38 per cent were males. These figures were derived from a number of different breeds and crosses, for each of which the sex ratio was higher than 50. As regards hatchery data, it should be noted that any errors usually favor the male sex, as doubtful cases are classified as males. Out of 515,976 living chicks that were sexed by plumage color (which is more accurate than palpation), 50.34 per cent were classified as males. Crew himself sexed 8565 dead-in-shell embryos from these latter settings and found 51.03 per cent to be males. He concluded that, if any sexual preference in prenatal mortality occurs, the male sex apparently suffers more. The data amassed by Crew contradict the majority of other reports, in finding an excess of males both among dead embryos and among living chicks. Such a situation suggests that the primary sex ratio (at time of fertilization) must be considerably higher than 50.

Hays (1945) attempted to determine the primary sex ratio by sexing all the progeny of dams that had given records during the breeding season of 100 per cent fertility and 100 per cent hatchability. He sexed 870 progeny from 39 purebred Rhode Island Red dams with such perfect records and obtained a sex ratio of 49.7 per cent. However, the sexing was done at the age of 8 weeks, and 61 chicks were lost before then; hence these data are not conclusive. Hays (1941a) previously had found a sex ratio of 50.85 per cent males out of 23,273 Rhode Island Red chicks sexed at 8 weeks. If one assumes that the sex distribution of chicks that died during the first 8 weeks are comparable in these two sets of data, a preferential prenatal mortality of the female sex is indicated at least for Hays' stocks.

Lambert and Knox (1926) found an excess of males among all sexable embryos dying before the eighteenth day of development.

tion for good vigor and high hatchability undoubtedly involves a great many different genes.

There is ample evidence that hereditary factors other than genetic lethals play an important role in determining the level of hatchability. It has been the common experience of all poultry breeders who have kept pedigree records that under identical management fowls differ greatly in the hatching quality of their eggs. Individual hens, furthermore, have been shown generally to maintain a fairly consistent level of hatchability throughout the season and during successive years (Pearl and Surface, 1909; Dunn, 1924; Hays and Sanborn, 1924; Snyder, 1931; Hyre and Hall, 1932; Jull, 1934). In addition, positive correlations ranging from 0.16 to 0.21 between the hatchability levels of related individuals have been reported: between sisters (Pearl and Surface, 1909) and between dams and daughters (Hays and Sanborn, 1924; Jull, 1931b; and Bronkhorst, 1933). And Hays and Sanborn (1924) found the coefficient of correlation between first- and second-year daughters of the same males to be 0.300 ± 0.086 . All these observations are indicative of the inheritance of hatchability.

The effectiveness of selection for high hatchability has been demonstrated by many investigations. Those concerned with inbreeding have already been discussed. Selection in linebred and noninbred flocks also has been used to improve hatchability. Hays and Sanborn (1924) reported that selection for 10 years of Rhode Island Red breeding birds from dams with good hatching power had resulted in a gradual improvement in hatchability of the flock from 59 per cent to 70.5 per cent. In 1939 the same investigators announced that continued selection, plus some improvements in incubation facilities, had further raised the mean hatchability of the general flocks to 86 per cent in 1938. Hays and Klein (1943) observed that, whereas in 1933 only 61 per cent of the females in this same flock attained a hatchability of 85 per cent, selection of breeding males and females from levels of 85 per cent or higher had resulted by 1940 in a flock in which three-fourths of the females hatched at least 85 per cent of their eggs.

Jeffrey (1944) obtained similar results with White Leghorn fowl. Selection was primarily for egg production, and secondary

Turkeys

Asmundson (1941) has also presented data for sex ratios in turkey embryos and poult's (mostly of the Bronze variety). He found an excess of males among dead turkey embryos at all stages of development. Prior to the twentieth day, the sex ratio of 515 dead embryos was 55.34 per cent; among 6867 embryos dying after the twentieth day or failing to hatch, 51.51 per cent were males. Among over 12,000 hatched poult's, the sex ratio was 49.2 per cent. Asmundson's data definitely indicate that, at least in his flocks, male turkeys suffer a higher embryonic mortality than female turkeys.

THE INHERITANCE OF HATCHABILITY; HATCHABILITY GENES

Lethal and semilethal genes have been shown to produce morphological or physiological changes that lead to the death of the embryo or to its inability to hatch. These effects on hatchability are definite and to a large extent calculable. It has also been noted that other mutations that are not actually lethal may decrease embryonic viability to varying degrees under differing circumstances. We have evidence of some genes that modify the effects of lethals and thus indirectly alter hatchability, as, for instance, the modifiers of the Creeper gene in the Japanese Bantam breed and of the short-upper-beak mutation. We have also seen that hereditary factors that determine the sex of the individual may have an indirect effect on its viability.

But, with the exception of these genes about which we have more or less information, we know little or nothing about the many other factors in the total genetic background which may affect hatchability. The complex interactions of the genes that control the physiological and biochemical processes of the developing embryo presumably may affect hatchability in many ways. Vigor, metabolism, growth rate, resistance to disease, and the ability to withstand given environmental conditions are all at least partially under genetic control. The survival of the developing embryo must depend upon the interaction between its total genetic background and its total environment. Successful selec-

abilities of daughters of other hens were very similar to their dams. Such variations occurred both in lines of high and of low hatchability. These results again seem to indicate that hatchability is not inherited in so simple a fashion as Hays and Sanborn have suggested.

Significant differences in hatchability levels between individuals and between different strains and breeds have been widely observed among turkeys as well as among chickens. Whitson, Marsden, and Titus (1944) found, for example, that the hatchability of eggs from a Broad Breasted Bronze strain was significantly lower during two successive years than eggs from Standard-bred Bronze, White Holland, and Beltsville Small White hens. Sampson and Wilson (1944) and Wilson and Johnson (1946) found that Beltsville Small White eggs hatched about 25 per cent better than fertile Bronze eggs in several successive years.

Also, selection of breeding turkeys has been shown to improve hatchability (Marsden and Knox, 1937). Asmundson and Lloyd (1935) observed that improvements in hatchability as a result of the selection of mated birds on the basis of their own or their dams' hatching records seemed to indicate a genetic influence.

Wilson and Johnson (1946) obtained an average regression coefficient of +0.13 for the hatchabilities of the daughters on the hatchability of the dams in the Bronze and Beltsville Small White varieties. They estimate that the heritability of hatchability in this population is 0.26 and conclude that selection of young hens from the highest 30 per cent of the dams and of toms from the top 10 per cent could improve hatchability 6.5 per cent in a single generation.

Although the available data are limited, it appears that hatchability is inherited in turkeys in much the same manner as in chickens.

The existence of specific genes for hatchability remains in doubt, although it is well established that hatchability is inherited. It seems most likely that the inheritance is in terms of gene complexes, or of multiple gene pairs, whose effects on hatchability are applied indirectly through their control of various physiological and metabolic processes. However, since both the environment and the total genetic background are so im-

ily for high hatchability and adult viability. An unselected control line also was maintained. The hatchability for the selected line increased from 66 per cent in 1936 to 78 per cent in 1938 and remained at a level of 78 per cent to 81 per cent through 1941. In the unselected line, on the other hand, hatchability gradually declined during the course of the experiment from 76 per cent in 1936 to 61 per cent in 1941.

Dunn (1921-1922, 1923c) had reasoned that, if differences in hatchability were due to general differences in constitutional vigor, then prenatal and early postnatal mortalities should be correlated. Unable to establish such a relationship, he concluded that independent sets of factors not associated with general vigor determined mortality before and after hatching. He did not believe that the same sets of deleterious factors were necessarily effective in different lines, however, as families appeared to segregate for specific differences in period and incidence of embryo mortality (Dunn, 1924).

Hays and Sanborn (1924) have advanced the only theory regarding specific hatchability genes. They suggest that a single pair of genes determines the level of hatchability. The dominant allele of this gene pair, *H* (for high hatchability), has a cumulative effect; in heterozygous condition it results in medium hatchability, in homozygous condition, in high hatchability (85 per cent or above). The recessive condition, *hh*, is responsible for poor hatchability (below 55 per cent). Females with hatching records above 85 per cent are all *HH*, according to the theory, but in order to express their potentialities they must be mated to high-hatching males, which are selected according to the hatchability levels of their dams and daughters.

Neither Jull (1931b, 1940) nor Bronkhorst (1933) considered that any investigation had yielded correlation values between dams' and daughters' hatching records that were high enough to confirm this theory of a monofactorial (single gene pair) basis for the inheritance of hatchability.

Snyder (1931) obtained very variable results with Barred Plymouth Rocks, in lines selected for high and for low hatchability. The majority of the pullets from a given hen segregated with respect to hatchability; some hens produced offspring whose hatchabilities differed greatly from that of the dam; the hatch-

secure. It may be due to a gradual deterioration in the composition of the eggs. There are fewer data available regarding the effect of the sire's age, but all indications are that the age of the male is of little if any importance, as long as sexual vigor and adequate sperm production are maintained. Possibly the age of the breeding stock may influence the incidence of embryo malformations, for Tur (1907) found that malformations were most frequent in the first pullet eggs.

Pearl and Surface (1909) kept hatching records during two successive years for 52 Barred Plymouth Rock hens. They found an insignificant improvement of 2 per cent in hatchability for the second as compared to the pullet year.

Hays and Sanborn have reported varying results from a flock of Rhode Island Red fowl. In 1924 they recorded that 253 birds having a pullet-year average hatchability of 56.8 per cent, hatched only 47.9 per cent of their eggs during the second year. The higher hatchability as pullets was significant. In later studies Hays (1928) and Hays and Sanborn (1928, 1939) found the situation to be reversed; two-year-old birds gave better hatchabilities than they had as pullets. The latest of these reports showed that over a period of 8 years mean hatchability of pullets was approximately 65 per cent; of two-year-olds, 75 per cent; and of older hens, 65 per cent. The final conclusion was that the best hatchability is obtained during the second breeding year.

Hyre and Hall (1932) found that 202 White Leghorn hens showed a 2 per cent superiority in hatchability for their pullet year as compared with their second year. Among 633 birds of the same breed, they observed hatching records of 61.4 per cent and 53.2 per cent in the second and third years, respectively. Hens from this same group which were kept as breeders for a longer period of time showed, in general, a gradual decrease in hatchability with each successive year. These data are included in table 42, broken down into groups for which records are available from the second through the third, fourth, fifth, and sixth breeding years. The combined data for smaller groups of Rhode Island Red, Barred Plymouth Rock, and White Wyandotte fowl are also given in the table; these showed a slight decrease in third-year hatchability, but a slightly higher hatchability in the second than in the first year.

portant, it would be extremely difficult to prove conclusively the activity of genes specific for hatchability even if they should exist.

INFLUENCE OF PARENTS ON HATCHABILITY

The hereditary constitution of the embryo is, as we have seen, of vital importance in determining its viability. The gene complex contributed by each parent is therefore, in all probability, the most important source of parental influence on hatchability. However, hereditary factors affecting the health, physiology, nutritional requirements, and vigor of the parents themselves may alter the hatchability of their progeny. This is particularly true of the dam, since the structure and chemical composition of the egg, which constitutes the immediate environment and nutrition of the developing embryo (see chap. 5), is a function of the dam's physiological condition and genetic makeup.

In earlier chapters, the effects on hatchability of various egg traits such as shell composition and color, egg size, weight, and shape, yolk composition, etc., have been discussed. All these characters are at least partially under genetic control. In addition, the rate of egg production, clutch size, time of day at which the eggs are laid, time intervals between eggs in a clutch and between clutches appear to be individualized characteristics that influence hatchability to a considerable extent. Landauer (1941c) and Bernier (1947) have presented extensive data and references on these subjects. Since these factors contributing to hatchability have been discussed elsewhere in this book (see chap. 2), they will not be considered here except to emphasize the fact that the genetic makeup of the dam through its control of egg and production characters is of vital importance in determining the hatchability of her offspring.

AGE

Numerous investigations have been made to determine the effect of the age of the breeding stock on the hatchability of the eggs. A progressive decline in hatchability with advancing age of the dam has been found. The cause of such a decline is ob-

ence. Nevertheless it is obvious that in these fowl hatchability was superior in the first laying year.

A general, progressive decline in hatchability up to an age of 7 years was likewise observed in most hens by Greenwood (1932). He also noted that the postnatal survival and later reproductive performance of chicks hatching from the eggs of very old hens was extremely poor. Five pullet daughters of old hens compared very unfavorably with their dams and with the earlier progeny of their dams in the hatchability of their eggs.

Funk (1934), Warren (1934), and Jull (1935) all observed that pullet eggs tend to hatch better than eggs from older hens of various breeds. Table 42 summarizes some of these data. Jull noted that, although the trend with ageing was obvious, the differences between successive years were not significant. Neel (1942) pointed out that both the percentage of hatchability and the rate of embryonic development tend to decrease with the age of the hen.

Although Martin and Insko (1934) presented evidence indicating that hatchability among selected birds did not decline with age, a continuation of the same study (Insko, Steele, and Wightman, 1947) brought these data into line with the findings of other investigators. Extensive records for both White Leghorns and Rhode Island Reds showed that hatchability did decrease with increasing age of the laying hens (table 42). With progressive ageing there was a proportionate rise in the percentage of weak chicks and in the embryonic mortality for each week of incubation. The data further indicated a positive relationship between productive longevity and hatchability. In general, the longer the period during which laying hens were able to meet the selection qualifications (for egg production, hatchability, viability of offspring, etc.), the higher the hatchability was found to have been in previous years.

The general decline which has been repeatedly observed in hatchability with increasing age of the dam is thus well established. However, in view of the conflicting reports regarding the superiority of pullets versus yearlings and in view of the small differences in hatchability between these two years, the safest conclusion appears to be that there is little if any deterioration in hatchability until the third laying year.

TABLE 42

EFFECT OF DAM'S AGE ON HATCHABILITY OF FERTILE EGGS IN CHICKENS
(Records for same hens in successive years)

Source	Breed	Number of birds	Per cent hatchability in breeding year								
			1	2	3	4	6	6	7	8	9
Hyre and Hall (1932)	White Leghorn	202	54	52
		633	..	61	53
		219	..	62	55	46
		85	..	62	69	58	47
		34	..	65	67	58	56	48
	Heavy (mixed)	124	49	52
		60	50	..	46
Axelsson (1932)	Mixed	33	68	47
Warren (1934)	White Leghorn	35	70	63
		12	75	66	70
		54	..	77	66
		23	..	78	72	61
		18	55	44
	Rhode Island Red	12	60	53	49
		32	..	64	63
		12	..	69	66	54
Jull (1935)	White Leghorn	21	80	74
		55	..	72	69
	White Leghorn and Rhode Island Red	20	..	71	75	74
		51	..	77	73
Insko, Steele, and Wightman (1947)	White Leghorn	52	87	82
		32	..	74	61
		71	..	78	71	62
		30	..	74	69	62	50
		24	..	67	54	63	66	46
		12	..	73	62	71	67	71	30
		4	..	68	50	75	91	67	68	71	..
		2	..	66	50	37	60	71	80	86	91
		20	89	83
		14	..	82	69
	Rhode Island Red	15	..	71	68	74

Axelsson (1932) found that hatchability was always higher among one-year-old hens as compared with two-year-olds for Rhode Island Red, Barnevelder, and White Leghorn fowl, and for crosses among these breeds. The average hatchability during the first year was 63.6 ± 0.77 per cent, and during the second year, 59.0 ± 1.27 per cent; the difference was considered significant. When the records of the same 33 hens as one- and as two-year-olds were compared, a difference of 21.5 ± 5.54 per cent was found (see table 42). Axelsson points out, however, that seasonal effects probably account for a large part of this differ-

There are no data indicating an effect of the age of the male turkey on hatchability.

In summary, then, we may conclude that after the second year of breeding, both turkey and chicken hens deteriorate with respect to hatchability of their eggs. Age of the sire is of little or no importance, as long as sexual vigor is retained.

HEALTH AND METABOLISM

The health and general metabolism of the dam frequently influence hatchability, presumably through their effects on egg composition. A number of examples may be cited.

Landauer and Bliss (1943) obtained consistent differences in the hatchability of eggs from reciprocal matings between heterozygous Creeper and normal fowl when the dams used for the comparisons were full sisters. Eggs from Creeper dams mated to their normal brothers averaged 5.3 per cent lower in hatchability than did those from normal dams by Creeper brothers. The differences in embryo mortality occurred during the last 5 days of incubation. Since the genetic composition of the progeny of these two types of matings was similar, it was concluded that there was a maternal effect of the dam's Creeper gene on the hatchability of her eggs. The investigators suggest that the Creeper bird is unable to store calcium adequately. This has no effect on the spermatozoa produced by the male, but it may have a very definite effect on the shell composition of eggs produced by Creeper females. Investigations proved that shell composition varied greatly in Creeper eggs, many shells being heavier and others lighter and thinner than normal. It is assumed that, since body storage of calcium is not adequate, the shell composition varies with the amount of calcium consumed. The abnormal shells from Creeper hens are, according to this hypothesis, the direct cause of an excessive embryo mortality.

A similar maternal effect, apparently, is associated with the Frizzle mutation. The gene for frizzled plumage is dominant, causing wavy feathers in heterozygous condition and curled feathers in homozygotes (Landauer and Dunn, 1930b; Hutt, 1930). Imperfect covering results in an increased metabolic rate (Benedict, Landauer, and Fox, 1932) and is associated with changes in

As we have noted, there is little available information concerning the effect of the sire's age on hatchability. Hays (1928) was unable to demonstrate a significant relationship between the age of the sire and the hatchability of his mates' eggs, although he obtained slightly higher records from matings with yearling males than with cockerels. Warren (1934) also found that hatchability was not affected by the age of the sire. Jull (1935) observed that there were no consistent differences between cocks and cockerels among Rhode Island Red fowl, but that White Leghorn yearling males were somewhat superior to cockerels. Again the difference was not significant.

Asmundson and Lloyd (1935) found the effect of age on hatching records of Bronze turkey females to accord with the trend for chickens. For the first 2 years, there was no difference in hatchability (table 43), but after the second year of production, a

TABLE 43

EFFECT OF DAM'S AGE ON HATCHABILITY OF TURKEY EGGS

(Only data from Marble and Margolf are based on records of the same birds in successive years)

Source	Breed	Per cent hatchability in breeding year				
		1	2	3	4	5
Asmundson and Lloyd (1935)	Bronze	52	51	38	38	19
Marble and Margolf (1936)	57	55
Scott (1937)	Bronze	73	55

marked decrease was observed. Marble and Margolf (1936), from data on 20 turkeys bred both as young and as yearling hens, reported that hatchability decreased an insignificant amount (viz., from 56.7 per cent to 54.8 per cent). Scott (1937), on the other hand, compared yearling hens with their daughters and found a very significant difference in hatchability in favor of the younger hens: 73.1 per cent as compared with 55.1 per cent. He concluded that young hens were superior as breeders. Hays and Klein (1943) state that hatchability is better when younger birds are used as breeders.

of eggs fertilized by different males. Bernier (1947), on the other hand, was unable to demonstrate the sire's effect by comparing inbred and outcross matings.

The influence of the sire and the dam is reflected in the correlation coefficients between hatchabilities of parents and offspring. Comparisons of the hatchability of dams and daughters by various investigators have yielded small but significant correlation coefficients ranging between 0.16 and 0.21 (Hays and Sanborn, 1924; Jull, 1931b; and Bronkhorst, 1933). Jull also demonstrated the dam's influence in another way. He separated the dams into two groups, one with hatching records above and one below the mean of the entire sample, and then calculated the mean hatchability for the daughters from each group. Daughters of the superior dams had a significantly higher mean hatchability than daughters of the inferior dams. The results are summarized in table 44.

TABLE 44

COMPARISON OF HATCHABILITIES OF DAUGHTERS FROM DAMS WITH HIGH AND LOW HATCHING RECORDS
(Adapted from Jull, 1931b)

Breed	Dams		Daughters	
	Number	Mean hatchability (Per cent)	Number	Mean hatchability (Per cent)
White Leghorn	36	81.24 ± 0.36	62	63.40 ± 1.02
	24	64.36 ± 0.76	43	52.33 ± 1.04
Rhode Island Red	51	80.63 ± 0.45	105	75.10 ± 1.29
	23	59.95 ± 0.78	43	66.36 ± 1.98

Direct comparisons of the male are not possible, and correlations must be measured in terms of the hatching records of their dams, mates, and daughters. Between dams and daughters of the same males Hays and Sanborn obtained a correlation coefficient of 0.059, and Bronkhorst of 0.092. Between dams and mates of the same males Hays and Sanborn obtained 0.158, and Bronkhorst 0.131. Hays and Sanborn also made the following comparisons: between mates and daughters, 0.227; between first- and second-year daughters, 0.30; and between sires' mates and sons' mates, 0.076. All these correlations are somewhat unreliable and

ogy, and reproductive activity of poultry, there are few data concerning any direct influence of either the maternal or the embryonic hormones on hatchability. Koch (1935, 1936, 1937) and Westermayer (1936) claimed that hatchability could be much improved by feeding follicular hormone to the laying hens; Prüfer (1936) and Ebbell (1938), who made extensive tests, were unable to substantiate this conclusion, and Simon (1939) found an increased hatchability in some, but not in most tests. Andrews and Schnetzler (1945) found that although thiouracil fed to hens was transmitted through the egg, causing a significant increase in the weight of the thyroid glands of the hatched offspring, there was no effect on hatchability. Scharrer and Schropp (1932) found that the feeding of 2 milligrams of iodine per day resulted in a definite increase in hatchability. An increased activity of the thyroid gland may have been involved.

Experimental procedures involving the extirpation of embryonic endocrine glands or the injection of hormones into the egg before or during incubation certainly affect hatchability considerably, often as a result of induced teratological development. By control experiments the amount of mortality attributable to the operative method *per se* can be separated from that caused by the removal or injection of the gland or its products. It is possible that natural hormonal differences between embryos or their dams may in similar manner be responsible for a certain percentage of embryonic mortality and teratological development. Scott (1937) observed that hatchability was significantly lower in the eggs from turkeys exposed to extra lighting. If this effect is not an artifact, it may be mediated by the dam's hormonal balance.

COMPARISON OF INFLUENCE OF SIRE AND DAM

Whereas the dam's genetic constitution and physiological condition exert a considerable influence on the hatchability of her eggs, the principal, and perhaps the only, contribution of the sire to hatchability is through the gene complex that his offspring inherit from him. Waters (1944) demonstrated this paternal effect by diallel crosses of the same females to different males. There were statistically significant differences in the hatchability

(1945) have been able to demonstrate histological differences between dead and infertile chicken eggs that had not been incubated, it must be accepted that early deaths are ordinarily classified as infertiles. Munro and Kosin suggest that these early deaths which occur during the first 24 hours after fertilization constitute a so-called peak of embryo mortality. These deaths may also explain, at least in part, the correlation between apparent fertility and hatchability which has been observed by Pearl and Surface (1909), Knox (1927), Jull (1928), Montemayor (1936), Munro and Kosin (1945) and Blyth (1945).

PREOVIPOSITAL DEVELOPMENT AND HATCHABILITY

Several recent studies have presented evidence which indicates that the developmental stage of the embryo at the time the egg is laid is of vital importance to that embryo's potential hatchability. Nicolaides (1933) and Hays and Nicolaides (1934) observed from a study of sectioned blastoderms of freshly laid, fertile eggs that the embryos in eggs from a given individual hen had a characteristic stage of development. Furthermore, the higher the hatching record of the dam, the more advanced was the embryo. These investigators found the most frequent stage of development in new-laid eggs from the best hatchers to be a well-advanced gastrula. Taylor and Gunns (1935, 1939) were unable to confirm the existence of an intimate relationship between stage of development at time of laying and potential hatchability, but they did find a positive correlation between the amount of overgrowth and hatchability. They also agreed that the stage of development expressed the dam's individuality. McNally and Byerly (1936) made somite counts after 48 hours of incubation and found a relationship between somite number at this stage and the hatching record of the dam. More advanced development paralleled increasing hatchability up to the optimum of 20 somites; a higher count appeared to be detrimental to hatchability.

Bernier (1947) observed that stage of development after 38 hours of incubation accurately reflects advanced or retarded development at earlier periods and can therefore be used to estimate

for the most part lower than those for dams and daughters. They substantiate the conclusion that the sire has less influence on hatchability than the dam.

However, there are some indications that the sperm as well as the egg can have a nongenetic effect on embryonic viability. It appears that stale sperm (at least, sperm more than 10 to 14 days old) results in a decrease in hatchability. This has been observed by Philips (1918), Crew (1926), Dunn (1927a), Warren and Kilpatrick (1929), and Nalbandov and Card (1943), among others. Nalbandov and Card reported also that with increasing age of the sperm (as determined by the time interval since the last mating) hatchability declines more rapidly than fertility, and the older the sperm the earlier the stage of embryonic death.

Morphological changes (that is, loss of flagellum, etc.) in the sperm cell were demonstrated to occur after a period of 24 hours by Warren and Kilpatrick. Such changes due to age may account for the fact that freshly introduced sperm takes precedence in fertilization over sperm from previous matings. Since senescence of sperm cells has been shown to lower embryo viability, it is hypothetically possible that other physiological differences between the sperm of different males may also affect hatchability.

Ordinarily, under conditions of good management, staleness of spermatozoa would probably be an infrequent cause of poor hatchability. It may be noted, however, that sexual discrimination against certain females on the part of the cock may reduce not only the fertility but also the hatchability of fertile eggs laid by such females.

EMBRYONIC GROWTH AND DEVELOPMENT

FERTILITY VERSUS HATCHABILITY

Fertility and hatchability are related problems. Our discussion of hatchability has been in terms of fertile rather than of total eggs set. However, it should be remembered that by ordinary methods of candling it is impossible to distinguish infertile eggs from fertile ones in which very early embryonic death has taken place. Even at the time of laying, some development, variable in amount, has occurred in a fertile egg. Although Munro and Kosin

in incubation temperatures have been observed to result in changes in growth rate and in accelerated or retarded hatching at a low-hatchability level (Barott, 1937; Byerly, 1938; Henderson, 1939; and Romanoff, 1939). It is probable that numerous environmental factors may influence hatchability through alterations of the embryonic metabolism and growth rate (Barott, 1937). Landauer (1941b) has suggested changes in growth rate as a possible factor in the production of distortions in development which lead to malformations and death.

PEAKS OF EMBRYO MORTALITY

Regardless of whether hatchability is high or low and whether natural or artificial means of incubation are employed, the distribution of embryo mortality falls into a specific and significant pattern. There are definite peaks of mortality, and, when hatchability is poor, these peaks are exaggerated (Bronkhorst, 1933). Under special conditions additional, but usually less significant, peaks appear. Payne (1919) first reported that 65 per cent of all embryo mortality occurs between the fourth and the sixth or after the eighteenth day of development, the latter period representing the peak of maximum mortality. Byerly (1931) corroborated Payne's observations in general, but he found that the first peak was somewhat higher and occurred slightly earlier than Payne had reported. The shift was probably due in part to the reclassification as early dead of a number of eggs that had been candled out as infertiles. In addition, Byerly noted that, since growth and expansion may continue in the extra-embryonic vascular area after the death of the embryo, Payne's method of determining age by the size of the blood ring would frequently result in erroneous estimates of the age at which the embryo died. Byerly also found a minor mortality peak at 10 days in some, but not in all, hatches. Munro and Kosin (1915) have proposed that a still earlier peak in mortality may be present before oviposition. Nutritional factors and sun-shine also may alter the pattern of embryo mortality (see chap. 1).

the degree of development at time of laying. From extensive experimental data he demonstrated a curvilinear relationship between blastoderm size at time of oviposition and hatchability. In effect, there is an optimum embryo size for optimum hatchability; this size is a function of many factors, including the genetic constitution of the dam, the time of day at which the egg is laid, the position of the egg in the clutch, the time interval since the previous egg was laid, and possibly the degree of inbreeding. Embryos that at oviposition are either more or less advanced than the optimum tend to hatch less well. It is probable that the explanation lies in the fact that the shock from being chilled and having development arrested at oviposition is most hazardous to the developing embryo at particular stages of development.

GROWTH RATE AND HATCHABILITY

Numerous observations on the growth rate of chicken and turkey embryos have suggested that there are changing cycles in the speed of growth. These changes in rate of growth are probably due to alterations in the metabolic processes during the course of development. Few studies have attempted to correlate growth rate under normal conditions with hatchability. Bronkhorst (1933), however, studied the growth of embryos from hens with high (above 75 per cent) and low (below 50 per cent) hatchability. He found that embryos from the high-hatching group exhibited an earlier maximum period of growth and a much greater weight increase at the end of incubation than embryos from the low-hatching group. The latter were more variable in weight and in growth increments. Whether these phenomena represent a cause or an effect of the degree of embryo viability is undetermined. Neel (1942) also reported that a high rate of development at early stages appears to be correlated with high hatchability. By 72 hours, a fairly uniform rate seemed to be attained. Both rate of development and percentage of hatchability decreased in older hens.

A retarded growth rate may be a factor in the delay as well as in the decline in hatching which both Dunn (1923b) and Byerly (1934) have observed among inbred chickens. Variations

further investigations are necessary to establish conclusively the exact nature of the changes and their relationship to breeding and inheritance, nutrition, and environment.

MALPOSITIONS

One phase of growth involves the successive assumption by the embryo of various positions during the course of development that ultimately produce the characteristic position for hatching; that is, orientation so that the longitudinal axis of the embryo corresponds to the long axis of the egg, the head is tucked under the right wing, and the tip of the beak points toward the air space at the blunt end of the egg. In this position, stretching movements finally bring the beak into contact with the shell, which is pipped as the embryo pivots around its long axis. Other positions hinder or prevent the embryo from hatching. These so-called malpositions have been classified into the six following groups which most investigators recognize:

Malposition I. Head between thighs.

Malposition II. Head in small end of egg.

Malposition III. Head towards or under left instead of right wing.

Malposition IV. Embryo rotated with beak away from air cell.

Malposition V. Feet over head.

Malposition VI. Beak above right wing.

Variations of these malpositions occur, and some embryos represent combinations of two of them. The fifth is uncommon and is usually a variation of other positions. Asmundson (1938) found no significant difference between chickens and turkeys with regard to incidence of malpositions.

Sanctuary (1925), Smith (1931), and Hutt and Cavers (1931) estimated that half of the embryos that die after the eighteenth day of development or that fail to hatch are in malpositions. This constitutes approximately one-fourth of the total embryonic mortality.

Sanctuary (*op. cit.*), Smith (*op. cit.*), Byerly and Olsen (1934b), and Asmundson (1938) have all suggested that genetic factors may be involved. Certain hens apparently produce more embryos in malpositions than other hens, and individual hens yield different frequencies of a given type of malposition. Upp

first week. Crossbreeding, on the other hand, has little effect on first-week mortality but greatly reduces the death rate during the last 3 or 4 days of incubation. The superior qualities of eggs from hybrid females were indicated by a definite lowering of the first mortality peak.

Bronkhorst (1933) found that the mortality distribution of embryos from hens with low-hatching records exhibited the same two main peaks (the earlier one being the more exaggerated) as the mortality distribution from high-hatching hens. Hall and Van Wagenen (1936), on the other hand, observed an additional distinct mortality peak on the sixth day among embryos from a selected White Leghorn line with low hatchability. This sixth-day peak was absent in both unselected White Leghorns and a line of the same breed selected for high hatchability.

A comparison of the mortality distributions of 961 chicken (White Leghorn) and 799 turkey (Bronze) embryos was made by Insko and Martin (1935). For their chick embryos, the peaks of mortality fell on the second and the nineteenth days, and for the turkey embryos, on the fourth and the twenty-fifth days. Mortality was uniformly low between these peaks. Thus, although the earlier mortality peaks were not completely parallel, they were close, and the second mortality peaks coincided according to the relative stage of development of each. Orlov and Kuchkovskaya (1941) reported that maximum mortality of turkey embryos from a high-hatching group fell on the third and the twenty-fifth days; in a low-hatching group there was also a mortality peak on the third day, but, in the second critical period, mortality continued to rise until the end of incubation.

The comparable critical periods at which death is most likely to occur in the development of both chicken and turkey embryos are matched by similar critical periods in the development of other birds (Riddle, 1930). Suggestions have been made regarding the possible importance of changes in the metabolism or the functioning of various organs and systems by Schmalhausen (1926), Brody (1927), Riddle (1930), Needham (1931), Priagle and Barott (1937), Ogorodniy (1939a, b), Noyons and Pascal de Hesselle (1939), and Seck (1941). The situation is exceedingly complex. Although it seems fairly certain that critical functional and metabolic changes must be taking place at these periods,

Differences in frequencies of malpositions also occur according to whether eggs are incubated horizontally or vertically with the large end up (Byerly and Olsen, 1931, 1934a, 1936b, 1937; Cavers and Hutt, 1934; and Hutt and Pilkey, 1934). Horizontal incubation results in increased frequencies of embryos in Malpositions II and IV, whereas vertical incubation produces an excess of Malposition III. Asmundson (1938) found that turkey embryos also exhibit an increased incidence of Malposition II when horizontal incubation is practiced.

Other phases of management affect the incidence of malposition. According to Insko and Martin (1933) frequent turning of the eggs reduces the frequency of malposition. Abnormally high or low incubation temperatures result in more malpositions (Byerly, 1938). Insko and Martin's data (1935) indicate that the type of incubator used for turkey eggs may affect the relative frequencies of the different malpositions. Improper diet of the laying hens, particularly a vitamin-deficient diet, also may increase the incidence of various malpositions (Smith, 1931; Byerly and Olsen, 1934b; Insko, 1934; Polk and Sipe, 1940).

All malpositions were at first assumed to be a direct cause of death or of failure to hatch. Further investigations have indicated that this is not necessarily true; some malpositions may be the result of death or delayed development (Byerly and Olsen, 1934b; Dove, 1935; and Byerly, 1938). Waters (1935a, b) made daily determinations of embryo position during the latter part of incubation, with the following very interesting results. On the eighteenth day, none of the embryos was in the normal position for hatching; the majority were in Malposition I, head between thighs. On the nineteenth day, approximately half were in the normal position, or in Malposition VI (the beak above instead of below the right wing), and the rest were in Malpositions I, II, and III. By the twentieth day, at least half of the embryos had assumed the normal position, the next largest group were in Malposition VI, and 16 percent or less were in other positions. Examinations of dead embryos showed that none was in the normal position before the twentieth day of incubation, whereas very few embryos dying on the twenty-first day were in malpositions. Waters thus demonstrated conclusively that embryos normally shift position after the eighteenth day. He also found

(1934c) reported that inbreeding raises the frequency of malpositions, and Asmundson (1938) discovered breed differences among chickens and individual differences among turkeys in the incidence of Malposition III. Individual differences in the characteristic size of eggs may be of importance in these respects, as Hutt (1938) has reported a definite increase in incidence of Malposition III among embryos in larger eggs. Byerly and Jull (1932) also found a tremendous increase of the same malposition among embryos homozygous for the lethal sticky gene. Byerly and Olsen (1934b) observed that in general, Malposition III is a reflection of adverse environment or an unfavorable genetic background, and it is often associated with small or obviously malformed embryos.

Many nongenetic factors have been shown to affect the incidence of certain malpositions, among them position of the egg during incubation. Byerly and Olsen (1931) observed that, when eggs are incubated with the small end up, more than half of the embryos assume Malposition II, the head in the small end of the egg. But if the small end is coated with paraffin when the egg is incubated with the small end up, the incidence of Malposition II is cut in half, although it is still considerably above normal. Incubation of the egg with the blunt end up and coated with paraffin also results in an abnormally high frequency of Malposition II. Apparently both gravity and air-hunger influence embryo orientation. Further studies led Byerly and Olsen (1933, 1936b) to conclude that both Malpositions II and IV are determined during the second week of incubation and that the adherence of the allantois to the shell membrane at this time may be a critical factor in fixing the embryo's position. Taylor (1932) and Cavers and Hutt (1934), however, found that abnormal orientation of the embryo in the egg as early as the fourth or the sixth day appeared to be correlated with an increased frequency of Malposition II. Normally oriented 6-day embryos hatched 5 per cent better than abnormally oriented embryos. Heterotaxia (embryos lying on the right instead of the left side), on the other hand, was found to be completely independent of the position of the egg, of malpositions, or of hatchability (Taylor, 1934; Olsen and Byerly, 1935).

studied. Such malformations of nongenetic origin account for a small and variable percentage of embryonic mortality in any large setting of eggs. In many malformations the cause of abnormal development is inexplicable; in others it has been possible to trace certain factors that contribute to the production of relatively high frequencies of a given type of malformation; and in still other malformations a direct environmental or nutritional origin has been found.

Some of the more commonly recurrent gross sporadic abnormalities have been described by Hutt and Greenwood (1929b) and Hutt (1931). Groups of associated defects are frequent. The head is most often affected. Reduction or absence of one or both eyes is frequently associated with unilateral facial reduction and a shortened, crossed, or deformed beak. Hernia of the brain which may be accompanied by other abnormalities such as a short upper beak and eye malformations is also common. Absence of the entire head and cyclopia (single median eye with associated defects) occur occasionally. Hutt reported that, in 17,771 unhatched eggs, there were 559 teratological monsters. Of these all but 31 had head abnormalities of some sort. Byerly (1931) found the incidence of terata among 2087 dead embryos to be 9.5 per cent, three-fourths of which had abnormalities of the brain or eyes. Eggs from reciprocal matings between White Leghorn and Rhode Island Red fowl contained fewer monsters than eggs from either of these two pure breeds (Byerly, 1930).

Other malformations that are found repeatedly include micromelia; absence or defects in structure of the limbs, digits, and tail; duplication of limbs or digits; partial or incomplete twinning; and ectopia or eversion of the viscera. Defective development of the heart and circulatory system, which may cause anemic embryos, is usually fatal at early stages of development.

One rather frequent sporadic malformation in chickens is chondrodystrophy, a form of micromelia with characteristic irregularities in cartilage formation and ossification of the long bones (Landauer and Dunn, 1926; Landauer, 1927; Dunn, 1927b). The gross morphological symptoms of shortened limbs, often accompanied by malformations of the skull, are indistinguishable from those of nonchondrodystrophic forms of micromelia. Several genetic types have already been discussed. In

changes in the position of the air cell after the nineteenth day. Byerly and Olsen (1937) likewise agreed that shifts in embryonic position occur during this period, and Asmundson (1938) reported that similar shifts occur with turkey embryos. The obvious interpretation is that few embryos die because they are abnormally oriented but that, as a result of death, the embryo may be in a so-called malposition which indicates the final stage of normal development attained. Waters considered the normal position at 18 days to be with the head between the thighs. In the transitional stages of shifting to the hatching position, the head may at first lie above the right wing and later be tucked beneath it. In fact Byerly and Olsen (1936b) have further suggested that Malposition I (the head between the thighs) may be a transitional position in shifting the head from left to right. The same authors suggest also that either embryos are able to shift from Malpositions I and IV to normal or they hatch from these malpositions on occasion (Byerly and Olsen, 1936a). Increased frequencies of malpositions under various unfavorable conditions may, according to this interpretation, merely indicate an abnormally delayed development, so that fewer embryos have shifted to the normal position at the end of the standard incubation period.

In any event, not all malpositions prevent hatching. It is difficult and sometimes impossible to determine what may have been the exact position of all the chicks that did hatch. Waters has pointed out, for instance, that we do not know how many chicks hatch from Malposition VI, beak above right wing. Dove (1935) considers this position a normal variant, and Byerly and Olsen (1937) suggest that both Malpositions V and VI are probably normal. A study of the position of embryos in pipped and unpipped eggs suggests that pipping, and therefore hatching, is almost impossible from Malpositions I and III. Pipping and hatching are possible from Malposition IV, and 46 per cent of 2130 embryos in Malposition II were able to hatch (Byerly and Olsen, 1936a).

TERATA

Many sporadic developmental malformations occur repeatedly, not only among poultry but also in other animals. As a result, various abnormal patterns and syndromes have been quite widely

found an increase, on the other hand, in eye deformities as the hatching season progressed. This was confirmed by Landauer (1943), who also reported a seasonal increase in sporadic rumplessness and duplications of various sorts. Temperature, humidity, and the amount of sunshine available are all possible factors that may contribute to the appearance of a seasonal influence.

Hutt and Pilkey (1930) found that the time of day at which the egg is laid also has an effect on the frequency of malformations. The incidence is lowest among eggs laid before 9 o'clock in the morning and rises gradually to reach the maximum among eggs laid after 2 P.M.

At least a part of the reduced hatchability that results from unfavorable environmental conditions during incubation is due to an increased frequency of malformations. Landauer (1941c), for instance, reported that improved incubation facilities at the Storrs Agricultural Experiment Station resulted in a decrease in average incidence of head abnormalities from 1.41 per cent to 0.22 per cent of all fertile eggs. Better control of humidity was considered the chief factor in this improvement.

Numerous experimental procedures have been found to increase the frequency or to produce duplicates of spontaneously occurring monsters. Phenocopies of both lethal and nonlethal mutations have also been produced. Such experiments have not only provided much information as to the mechanics of the development of embryonic malformations but they have also occasionally promoted suggestions of possible natural causes and modes of origin of these abnormalities. Since physiological gradients have been demonstrated in chicken embryos (Buehanan, 1926; Hinrichs, 1927; Hyman, 1927; Tazelaar, 1928; and Rulon, 1935), it is probable that growth distortions and consequent malformations may arise from the action of either depressing or overstimulating environmental conditions. Other factors may upset specific organizing centers at critical periods, with resultant defects in related parts. Some agencies may simultaneously interfere with a particular metabolic or physiological process throughout the embryo and thus produce a characteristic syndrome of abnormalities.

Some of the experimental methods that have been employed in these studies include the removal or transplantation of various

addition, a number of nongenetic forms of micromelia have been found to be of nutritional origin. Deficiencies in riboflavin (Ogorodniy, 1939a; Romanoff and Bauernfeind, 1942), biotin (Cravens, McGibbon, and Sebesta, 1944), and manganese (Lyons and Insko, 1937) in the diet have all been reported to result in high frequencies of chondrodystrophy, with a consequent decline in hatchability. Until histological examinations of the cartilage differentiation in the micromelias caused by vitamin deficiencies have been made, it would be preferable to classify them as micromelia, rather than chondrodystrophy. Landauer's study (1936) of the short-limbed embryos from manganese-deficient diets has shown that these, at least, are not representative of true chondrodystrophy. Patton (personal communication) also is of the opinion that the malformation caused by manganese deficiency is not chondrodystrophy. Excess sulfur (Holmes, Halpin, and Herrick, 1941) in the diet also causes similar morphological abnormalities. Extensive studies of the micromelias caused by genetic, nutritional, and other factors will be necessary in order to classify them in related groups according to microscopic anatomy and probable mode of origin.

The cause of the sporadic occurrence of true chondrodystrophy remains unknown, although various factors are known to contribute to its frequency. Dunn (1927b) observed that certain dams produced a much higher percentage of chondrodystrophic offspring than other hens, but he was unable to establish any genetic basis for this observation. Those dams that for a given period produced numerous chondrodystrophic offspring often, later in the season or in subsequent years, failed to have any chondrodystrophic chicks. Age of the dam was not a factor. Dunn did observe a definite and gradual seasonal decline in the incidence of chondrodystrophy with advancing spring, which was later confirmed by Hutt and Greenwood (1929a), Muaro (1932), Smith (1934), Otrygaa'ev (1938), and Landauer (1943). Disturbances in maternal metabolism, possibly caused by such factors as sunshine, nutrition, or hormonal imbalance, may play a part by upsetting the normal chemical constitution of the egg.

Other malformations of chicken embryos also show seasonal trends in incidence. Smith (1934) reported a decline in the frequency of anemic embryos as summer approached. Upp (1934b)

defects of her eggs, may thus indirectly cause a lowering in hatchability. On the other hand, the only uncontestedly proved influence of the sire on the viability of his progeny consists in the gene complex that the offspring inherit from him.

We have seen that the rate of embryonic growth is not constant and, further, that there are critical periods during development at which death is more likely to occur. Unfavorable conditions exaggerate the two established mortality peaks, which occur during the first few and last few days of embryonic development. Genetic and environmental factors are known to alter the growth rate and mortality peaks, but the physiological processes involved remain obscure.

Embryonic malpositions are found very frequently among unhatched eggs. Although many of these malpositions may be a direct cause of failure to hatch, others are merely indicative of retarded development or of death at a particular stage of development—the embryo's position being normal for its stage. Probably most malpositions are symptoms of unfavorable inheritance or environment.

The frequency of embryonic malformations that are not due to genetic factors is also, to a large extent, indicative of the adequacy of the total environment, including both the egg and the incubation conditions. Some terata have been traced to definite causes; others, for want of causative agents, are termed sporadic or accidental abnormalities.

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regions and organs at different stages of development; the injection of inorganic and organic substances into the air space or yolk, either before incubation or at a later stage of development; and the localized application to the developing embryo of chemical substances, electric currents, X rays, or mechanical injuries. A discussion of the great number of experimental reports on this type of work is beyond the scope of this review. Suffice it to say that these experiments are of vital importance in building up our understanding of the fundamental processes of normal, as well as abnormal, development. For further information the reader is referred to Darcste (1891), Rabaud (1914), Wolff (1936), and Landauer (1941c).

SUMMARY

Hereditary factors influence the physiology of development and hatchability in many ways. At present, there are nineteen identified lethal and semilethal mutations among chickens and three among turkeys. These form an effective bar to hatching either for all or for a large proportion of the affected embryos. A great many other mutant genes also reduce hatchability to a lesser degree. Inbreeding and outbreeding practices have been shown to alter hatchability, presumably through the formation of favorable or unfavorable gene combinations. Even the sex of the embryo may influence its chance of hatching. Very little is known about the many genes that control all the physiological and metabolic processes of development and their probable indirect effects on hatchability. The general consensus is that the existence of genes specific for level of hatchability has not been proved. Rather, it seems probable that in the absence of definite lethal and semilethal genes, the influence of the embryonic genotype on hatchability is based upon the interactions between the entire gene complex and the particular environmental conditions.

The hereditary constitution, age, metabolism, nutrition, and physiological condition of the dam are of vital importance to the hatchability of her fertile eggs. All these factors participate in determining the physical and chemical structure and content of the various parts of the egg, which in turn provide the immediate environment and source of metabolites for the developing embryo. Any agency that, acting through the dam, results in

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The importance of the breeding flock as a source of infection and subsequent cause of mortality in chicks was first emphasized when it was discovered by Rettger and Stoneburn (1909) and Rettger, Kirkpatrick, and Stoneburn (1912) that pullorum disease (then known as bacillary white diarrhea) could be transmitted by infected eggs laid by carriers of the disease. Pullorum disease has continued to be of primary importance, because of the ease with which it is transmitted in the incubator (Hinshaw, Upp, and Moore, 1926). Other diseases are also of importance but have not been so costly to the hatching industry. Because of this fact, pullorum disease will of necessity receive major attention in this chapter. Other diseases of the breeding flock will be discussed in relation to subsequent chick and poult mortality, and methods for their control in the flock and in the hatchery will be reviewed. For more detailed information, the reader is referred to standard textbooks such as Biester and Devries (1943); Barger and Card (1943); Reis, Nobrega, and Reis (1936); Lesbouyries (1941); State Experiment Station bulletins; and to scientific reports referred to in the foregoing and in this chapter.

INFECTIOUS DISEASES OF THE BREEDING FLOCK

PULLORUM DISEASE

This disease, which is caused by *Salmonella pullorum*, produces high mortality in chicks and poult and is well known because of its economic effect on the hatchery business. Its cycle of infection, including localization in the reproductive organs of females and subsequent transmission to the chick or poult through the egg, is well known (see fig. 76). Evidence that it is transmitted in the incubator, first reported by Hinshaw, Upp, and Moore (1926) and confirmed by Hinshaw, Scott, and Payne (1928) and Bunyea and Hall (1930), stimulated research on methods of control in hatcheries which resulted in numerous papers on fumigation by means of formaldehyde (Gwatkin, 1927; Bushnell, Payne, and Coon, 1929; Graham and Michael, 1937; and Insko, Steele, and Hinton, 1941). Attempts were made by hatcherymen to substitute disinfection procedures for testing programs which were already well developed in the eastern United States, but such pro-

CHAPTER 8

Diseases in Relation to Hatchery Operations

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have pullorum disease should reject eggs from these infected flocks. Replacement stock for such flocks should be purchased from disease-free sources. This procedure has proved to be an economical method by which a hatchery can eliminate pullorum disease from its supply flocks and from the hatchery.

The National Improvement Plans for chickens and turkeys, described in *United States Department of Agriculture Miscellaneous Bulletins* 300 (chickens) and 555 (turkeys), provide for certification of flocks according to their Pullorum Disease Status and furnish an example of methods for conducting eradication programs. In other countries other methods of certification are employed, but the principles involved are the same. Under these plans in the United States a flock of chickens may be certified as *U. S. Pullorum Clean* after the entire flock has been tested by the agglutination test and no reactors have been found for two or more successive seasons; as *U. S. Pullorum Passed* if the flock is tested and no reactors are found; as *U. S. Pullorum Controlled* if less than 2 per cent of the flock is found to be reactors. At the end of the 1948-49 hatching season the *U. S. Pullorum Tested* class will be deleted from the plan. During the 1948-49 hatching season a tolerance of 3 per cent reactors was allowed.

This system of certification applies also to turkeys, except that a *U. S. Pullorum Clean* flock is defined as one that contains no reactors on the initial test and a *U. S. Pullorum Passed* flock is defined as one in which infection was found in the first test of the season but from which the disease has successfully been eliminated as proved by repeated testing before the hatching season starts. The controlled class is identical with that used for chickens. The *U. S. Pullorum Tested* class for turkeys was eliminated at the end of the 1947-48 hatching season. For details regarding the National Poultry and Turkey Improvement Plans as they apply to an individual state, the reader is referred to the Official Improvement Plan Agency in his state. If the name of this agency is not known, the local County Agricultural Agent's office will be able to supply the information. In other countries application to the comparable agency should be made for similar information.

cedures have now been proved to be adjuncts of rather than substitutes for the agglutination test and eradication programs. Fumigation has become a standard procedure for the disinfection of incubators between hatcheries, and, under certain conditions, during hatching (see section on omphalitis).

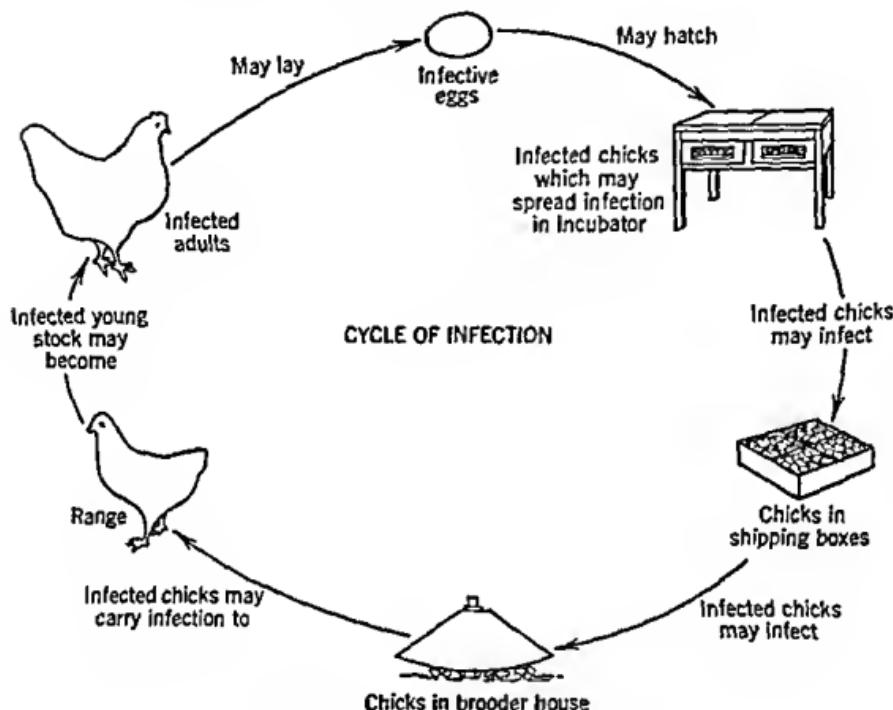


FIG. 76. Pullorum disease in a flock may be propagated from year to year in the manner illustrated in this figure. *Courtesy H. Van Roekel.*

Pullorum disease can be eradicated from breeding flocks, and the disease can thus be prevented in both chicks and poultts. To insure freedom from the disease, flocks should be tested by means of the agglutination test, and those found to be infected should be eliminated from the breeding program. Hatcheries that buy eggs from a number of flock owners can be certain of selling only pullorum-free chicks or poultts if all the supply flocks are free of the disease (that is, certified as U. S. Pullorum Passed, or U. S. Pullorum Clean, according to the National Improvement Plans for chickens and turkeys, or the equivalent of such certification). A hatchery that finds that a small percentage of its supply flocks

prospective buyer of chicks or pouls the correct information on the stock he is buying as far as their freedom from pullorum disease is concerned. The *U. S. Pullorum Clean* class is the highest grade and guarantees that the chicks or pouls came from pullorum-free breeding flocks. The *U. S. Pullorum Passed* class for chickens is equivalent to the *Clean* grade except that the flock has been certified as pullorum-free for only one year. Chicks from flocks or hatcheries certified as either *Clean* or *Passed* are the "top grades" as far as freedom from pullorum disease is concerned.

U. S. Pullorum Passed turkey pouls are a reasonably good buy because the flock has been tested until no reactors have been found. However, such pouls should not be purchased for breeding-flock replacements. *U. S. Pullorum Clean* turkey pouls are "top grade" and are the best buy for any purpose including breeding-flock replacements.

U. S. Pullorum Controlled and *U. S. Pullorum Tested* classes are inferior grades as far as pullorum disease is concerned, and they actually represent stock that comes from infected breeding flocks and infected premises.

The *U. S. Pullorum Controlled* or *Tested* classes for a hatchery may not indicate accurately to what extent the supply flocks of that hatchery are free from the disease, owing to the fact that a hatchery must be certified according to the grade of its poorest flock. For example, if all the flocks except one are free from the disease and that one flock has 1.5 per cent reactors, the rating for the hatchery would have to be *Pullorum Controlled*, if eggs from it are to be used. It is true, however, that as long as even a few supply flocks are infected there is danger that the disease may be transmitted in the hatchery. Hatcheries should, therefore, make every effort to eliminate such infected flocks and to furnish disease-free replacements so that the grades of the flocks can be improved.

For a more comprehensive discussion of pullorum disease, testing methods, and eradication procedures, the reader is referred to accounts of this disease by Van Roekel (1943), Hinshaw (1943a, b), and Barger and Card (1943). A monograph on pullorum disease by Rettger and Plastridge (1932) covers the literature to that date. No attempt has been made to discuss the three methods for agglutination testing, because most hatcheries are

now operating under some Official Testing Program that governs the procedure in the state in which it operates.

Recently, sulfonamides have been given much publicity as a means of controlling outbreaks of pullorum disease in chicks and poult. Severens, Roberts, and Card (1945), Anderson (1946), Mullen (1946), and Bottorff and Kiser (1947) have shown that mortality from pullorum disease in poultry can be reduced by sulfonamides when they are given at the rate of 0.25 to 0.5 per cent in the mash for periods up to a week in length. Drugs tested by these investigators included sulfamerazine, sulfadiazine, and sulfamethiazine. The prevention of all losses has not been reported. Contrary to early impressions from results obtained by Severens *et al.* (*op. cit.*), carriers are not eliminated by treatment with sulfa drugs, and therefore such drugs are not a substitute for a testing program. Treatment with sulfa drugs should be given only after a positive diagnosis of the disease has been made, and then only upon the advice of a competent veterinarian. Infected flocks that have survived after treatment should not be used for breeding purposes. The results of the experiments of Bottorff and Kiser (*op. cit.*) indicate that survivors of treated flocks may contain more carriers than survivors of untreated flocks.

In planning a prevention program it is very important to keep in mind that many animals other than chickens and turkeys may be carriers of pullorum disease. Van Roekel (1943) lists other birds as having been reported to have had natural infections of pullorum disease as follows: ducks, guinea fowl, pheasants, quail, sparrows, European bullfinches, and pigeons. He lists the following as having been reported vulnerable to infection by artificial inoculation: canaries, goslings, turtle doves, gold finches, and bitterns. The original references to these isolations are cited by Van Roekel. Edwards (1945) has reported another acute natural outbreak of pullorum disease in canaries, and Cass and Williams (1947) have isolated *S. pullorum* from a wild pheasant.

Mammalian species from which *S. pullorum* has been isolated include rabbits (Olney, 1928); guinea pigs, kittens, and young rabbits (Rettger, Hull, and Sturgis, 1916); foxes and minks (Benedict, McCoy, and Wisnicky, 1941); swine (Edwards and Bruner, 1943); and calves (Bruner and Edwards, 1946). The

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outbreak in rabbits, reported by Olney, followed the feeding of infertile incubated eggs obtained from a commercial hatchery.

Since 1940, the disease has assumed importance from the standpoint of public health because of reported isolations from man (Edwards and Bruner, 1943, and Mitchell, Garlock, and Broh-Kahn, 1946). In the outbreak reported by Mitchell et al., 433 persons developed gastroenteritis; the source of the infection was traced to a rice pudding containing eggs. The relationship of pullorum disease in fowl to public health further emphasizes the need for continued efforts to eradicate the disease from breeding flocks of chickens and turkeys.

FOWL TYPHOID

Fowl typhoid is caused by *Salmonella gallinarum* and generally affects chickens and turkeys after the brooder stage of development. It can, however, be transmitted in the hatchery and young chicks and poult may suffer heavy mortality. Probably the first description of losses in baby chicks was given by Taylor (1916) in a report on an outbreak of the disease affecting chickens of all ages. Beaudette (1925) and Beach and Davis (1927) reported on the disease in chicks and gave evidence that it was transmitted by adult carriers by tracing the chick losses to infected flocks of adults containing ovarian carriers of the causative organism. Beaudette (1930) in a paper presented at the Eleventh International Veterinary Congress summarized the literature on the disease to that date and reported for the first time the isolation of *S. gallinarum* from a dead chick embryo which originated from an infected flock. This isolation completed the evidence that fowl typhoid has a cycle of infection similar to that of pullorum disease. The writer has an unpublished record of the isolation of *S. gallinarum* from 17 of 23 eggs laid by a Rhode Island Red carrier hen from February 24 to May 3, 1935, when its last egg was laid. When the hen was killed on May 23, *S. gallinarum* was isolated from the ovary, oviduct, and peritoneal fluid. This record confirms Beaudette's findings that the disease can be transmitted through the egg. In turkeys the disease has a similar cycle of infection (Hinshaw and Taylor, 1933; Johnson and Pollard, 1910; and Boney, 1917).

Fowl typhoid has not become as serious a hatchery-disease problem as pullorum disease but, in areas where it is prevalent in either chicken or turkey flocks, hatchery owners must avoid using infected breeding flocks as sources of hatching eggs. It is especially important to eliminate from the breeding program flocks that contract the disease. Since the same agglutination test employed to detect carriers of pullorum disease will also detect carriers of fowl typhoid, using eggs from pullorum-free flocks will aid in preventing losses from fowl typhoid. Since, however, fowl typhoid is primarily a disease of adult birds, an outbreak may occur after a flock has been tested and pronounced pullorum-free. In that event the infected flock should be removed immediately from the list of supply flocks.

Bacterins containing *S. gallinarum* are not recommended for the prevention and control of fowl typhoid because they have not been proved of sufficient value in reducing losses. Also, bacterins interfere with the efficiency of pullorum-disease-eradication programs, because, according to Runnels and Thorp (1927), birds that have been given bacterins react to the agglutination test for as long as 36 days after a single injection and for at least 65 days after a double injection.

Studies by Hammond (1945), Moore (1946), and Holtman and Fisher (1947) indicate that sulfonamides may prove valuable in reducing losses from fowl typhoid in chickens if they are given before the disease becomes too widespread. None of these drugs, however, will eliminate the carrier problem.

There is evidence that birds saved by treatment may, on recovery, still carry the organism; therefore, survivors of treated flocks may have even more carriers among them than survivors of untreated infected flocks. Much attention is being given to the control of fowl typhoid as well as other diseases of poultry by drugs. It is to be hoped that these researches will discover a drug that will eliminate the causative organism completely and thus avoid the carrier problem.

The importance of sanitation in the prevention and the control of this disease on a community-wide basis is emphasized in a circular by Hall (1946). He outlines nine steps in a suggested community sanitation program, which can be applied equally well to many other diseases, as follows:

If fowl typhoid is to be controlled on a community-wide basis the following objectionable practices which contribute to the perpetuation and spread of fowl typhoid . . . should be avoided:

1. Dumping of dead birds on field or in woods, without burial.
2. Feeding of dead birds to hogs, dogs, cats, or other domestic animals. Partly consumed carcasses left around are fed upon by rats, buzzards, and other scavengers. These animals may then travel to a clean poultry flock and contaminate their feed or water with typhoid germs, and thus start a new outbreak.
3. The practice of chicken buyers bringing dirty crates into a clean yard or house and allowing the manure to fall on the ground is highly dangerous. These crates may have previously contained chickens which were sick with fowl typhoid.
4. Chicken buyers frequently drive directly into the broiler-house yard to load chickens, and the tires may be caked with mud from a yard where fowl typhoid was prevalent. This contaminated mud dropping off into a clean yard might start an outbreak in the next brood of chicks.
5. The dumping of poultry offal from dressing plants into fields, or into streams is a dangerous practice which might well be a means of spreading fowl typhoid. Streams contaminated with such material may carry typhoid germs a considerable distance to where the water may be drunk by chickens or carried onto clean premises by ducks or geese.
6. Broiler raisers often collect birds dead of disease and bring them all to the feed room where they may be thrown on the floor and cut open, thus contaminating the floor with blood, litter, and droppings which may be carried on the feet of the attendant to other pens which are free of the disease.
7. The return of feed bags to the dealer and re-use of dirty bags may spread disease, since empty feed bags are sometimes used to collect dead birds or may be contaminated by contact with dead birds in the feed room.
8. Vaccinating or testing crews may carry disease from contaminated premises to clean premises if proper care is not exercised to clean and disinfect footwear, change outer clothing, and clean and disinfect instruments and equipment such as bleeding knives or needles, tables, buckets, crates, and other equipment.
9. Dumping egg shells and dead-in-shell embryos in the fields or woods may cause fowl typhoid to be spread through the depredations of wild or domestic animals.

To this list should be added the warning already given that hatchery owners should never use flocks known to be infected as sources of hatching eggs. These precautions apply equally to chickens and turkeys.

There is evidence that the causative organism of fowl typhoid may live in the soil in some areas for as long as a year (Gauger,

Saphra, and Wassermann (1943) and for 65 per cent of the human cases reported in a European survey by Kauffmann (1941). It is also the type most frequently isolated from many other animals. These facts indicate the wide distribution of the *Salmonella* and emphasize the complexity of any prevention program.

Hatchery transmission is an important means of dissemination, although animal reservoirs are equally important. Evidence that this group of diseases may be transmitted through the egg in a manner similar to pullorum disease has been presented by Lee, Holm, and Murray (1936), Cherrington, Gildow, and Moore (1937), Pomeroy and Fenstermacher (1939), and Hinshaw and McNeil (1943). These investigators have shown that the disease may be transmitted through the egg, that adult carriers develop after an outbreak, and that the disease can be hatchery transmitted. The possibility of incubator transmission is illustrated by the following example taken from unpublished records of a co-operative project of the University of California and a commercial hatchery. Two lots of eggs, one from a flock known to be infected and one from a flock known to be free of infection, were hatched in separate machines. At the time of hatching, samples of poult down, removed from the hatching trays, were cultured for the presence of *Salmonella*. Down collected from the hatcher of the infected flock yielded the organism, whereas down from the hatcher of the noninfected flock was negative. Poulets from each lot were brooded separately, and *S. typhimurium* was isolated from several poulets that were exposed to the infected down and died within the first week. No cases of salmonellosis developed in the control group. Cultures of the organism isolated from the down also produced the disease when they were given to poulets.

Schalm (1937) and Pomeroy and Fenstermacher (1941) showed that eggs may become infected through the contamination of the shell by infected intestinal contents. Hinshaw and McNeil (1943) found that 81 per cent of *S. typhimurium* carriers yielded the organism from their intestines, as compared to only 17 per cent yielding it from the reproductive tract. The observations of these investigators thus indicate that, in this type of *Salmonella* at least, fecal contamination of the egg shell may be even more important as a means of infection than infection in the reproductive tract.

The use of the agglutination test as an aid in detecting and eradicating paratyphoid has been discussed by Hinshaw and McNeil (1943, 1944). The problem of testing is much more complicated than for pullorum disease, because of the greater complexity of the antigenic structure of the organisms involved. The multiplicity of the strains of *Salmonella* causing losses in birds further complicates the testing problem. Generalized testing programs will probably never be feasible, but a hatchery may find it desirable to use this means as an aid in eliminating a particular type of *Salmonella* from its supply flocks. The success of such a program will depend largely on the intelligent use of the information available on the antigenic nature of the *Salmonella* group. It is not a job for the amateur laboratorian.

Many hatcherymen and producers have the misconception that the agglutination test for pullorum disease will act equally well as an aid in an eradication program for the other *Salmonella* types. This is not generally true. The test will detect a high percentage of carriers of the members of Group D and a small percentage of the members of Group B of the Kauffmann-White classification as described by Edwards and Bruner (1942). For example, Hinshaw and McNeil (1943) reported that approximately 25 per cent of the total number of carriers of *S. typhimurium* were detected by the agglutination test with *S. pullorum* antigen. Some of the flocks included in their survey, which were known to be infected with *S. typhimurium*, failed to yield any reactors to the pullorum test. Therefore, the pullorum test should not be used as a "detection" test for this group of diseases. As

mortality, when given at the rate of 0.25 to 0.5 per cent in the mash for periods not exceeding 1 week. Pomeroy *et al.* called attention to the fact that the sulfonamides are, in general, more toxic for poult than for chicks. As in the case of pullorum disease and fowl typhoid, these drugs have not proved of value in the elimination of carriers from an infected flock.

Many manufacturers of biological products are promoting the use of bacterins for immunizing turkeys against salmonellosis. Most of these products contain only 1 or 2 of the 59 types of *Salmonella* which have been reported from turkeys. Such bacterins, if they proved to be of value, would give protection against only the specific types (or, at best, specific groups) used for making the bacterin, and the birds would still be susceptible to all the other types. Experiments under controlled conditions made by the writer and his colleagues (unpublished) have demonstrated the ineffectiveness of vaccination of day-old poult for *S. typhimurium* infection. Moreover, bacterins which are prepared improperly may occasionally be responsible for the introduction of disease into a community.

Essentials for prevention of this group of diseases are: (1) the elimination of flocks known to be diseased as sources of stock for breeding flocks; (2) separate hatching facilities for eggs from *Salmonella*-free flocks for the replacement of breeding flocks; (3) the frequent use by poultrymen of well-equipped diagnostic laboratories manned by adequately trained diagnosticians to discover new outbreaks which may endanger future replacement sources; (4) the recognition that there are numerous animal reservoirs of these diseases, which must be controlled to prevent transmission; (5) control of flies in hatcheries and brooders; and (6) the continuous co-operation of growers, hatcheries, veterinarians, and state agencies with breeders to secure replacements from salmonellosis-free sources.

TUBERCULOSIS

It is the general consensus of investigators that the egg or the hatchery is of only minor importance in the transmission of avian tuberculosis. Comprehensive reviews on this subject are given

by Feldman (1938) and Biester and Devries (1943). Feldman refers to several reports in the literature on the artificial transmission of tuberculosis to chicks by the inoculation of fertile eggs. Chicks so infected seldom live more than 1 month and the majority of them die from acute tuberculosis within 2 weeks after hatching. The hatchability of artificially infected eggs is always poor. Wahbi (1929) found that 6.2 per cent of 81 eggs laid by tuberculin positive hens were infected with tubercle bacilli. He also reported that, though the hatchability of artificially infected eggs is poor, such eggs may hatch, but the chicks therefrom soon die from tuberculosis. Fitch, Lubbehusen, and Dikmans (1924) and Fitch and Lubbehusen (1928) found that less than 1 per cent of eggs laid by tuberculous hens contain viable tubercle bacilli. They reared hundreds of chicks hatched from eggs laid by naturally infected hens without observing a single case of egg or hatchery transmission. Similar observations have been reported by Harshfield, Roderick, and Hawn (1937).

In our own studies on this disease in turkeys we found a higher incidence of infection of the reproductive tract than has been reported for chickens (Hinshaw, Niemann, and Busic, 1932). Including the cases of infection recorded in that report, we have observed 24 cases (42.1 per cent) of tuberculosis of the ovary in 57 autopsies of turkey hens. Only two cases of tuberculosis of the oviduct were found in these birds. One of the 5 males examined had tuberculosis of the testes. The only organs that showed a higher incidence than the ovaries were the liver (95.9 per cent), the spleen (75.3 per cent), and the intestine (57.6 per cent). Attempts to obtain eggs from tuberculous hens failed, and on autopsy the ovary was usually found to be nonfunctional. A total of 11 eggs laid by 2 tuberculous turkeys failed to produce the disease when chickens were inoculated with samples of the eggs, and no tubercle bacilli were found in the eggs.

Tuberculosis, even though its transmission is of minor importance as a hatchery problem, may cause severe losses through lowered egg production in breeding flocks and therefore should not be tolerated by hatchery owners. Flocks known to be tuberculous should be eliminated from breeding programs. For methods of prevention and control the reader is referred to standard textbooks on poultry diseases and to Feldman (1938).

RESPIRATORY DISEASES OF CHICKENS

With the possible exception of Newcastle disease (avian pneumoencephalitis), which is discussed under a separate heading, respiratory diseases (infectious laryngotracheitis, infectious bronchitis, infectious coryza) have not been proved to be transmitted through the egg. They may, however, be transmitted in a hatchery that has become infected by mechanical means. The transmission of these infections in the hatchery is usually associated with started-chick business or with a breeder flock and hatchery operated on the same ranch. The problem is discussed in greater detail under the heading incubator and hatchery sanitation (p. 363).

The proof that respiratory diseases of poultry are air borne seems imminent. DeOme (1947) has shown definitely that infectious laryngotracheitis may be transmitted by exposing susceptible chickens to dust-borne virus for as short a time as 15 minutes. There is little doubt but that future researches will prove that this method of transmission is an important means of disseminating many of the respiratory diseases of poultry.

Infectious Laryngotracheitis

Evidence that infectious laryngotracheitis is *not* transmitted through the egg is presented by Komarov and Beaudette (1932) and Brandly and Bushnell (1934). Komarov and Beaudette could not demonstrate the causative virus in the ovaries of birds either during the incubation period or at the height of the disease. Brandly and Bushnell found that the virus, when placed within or on the surface of eggs, does not seem to survive except for short periods under incubation environment. Normal chicks that they hatched from carrier hens did not develop the disease, and eggs laid by chickens soon after an outbreak did not contain the virus.

As may be expected of a disease which affects a flock so acutely, there is a marked drop in egg production during and after an outbreak of infectious laryngotracheitis. Figure 77, taken from a report by Hinshaw, Jones, and Graybill (1931) illustrates this effect. The chart was prepared from data collected on 25 acute

outbreaks in laying flocks. In these flocks it took 9 days to 6 weeks for production to return to the preinfection level. Similar results were obtained by Scott and Brandly (1934). Hatchability of eggs is likewise affected, according to these investigators. Smith (1933) found that even a mild attack of the disease results in a lowered hatchability which is reflected in increased embryonic mortality during the first and second weeks of incubation.

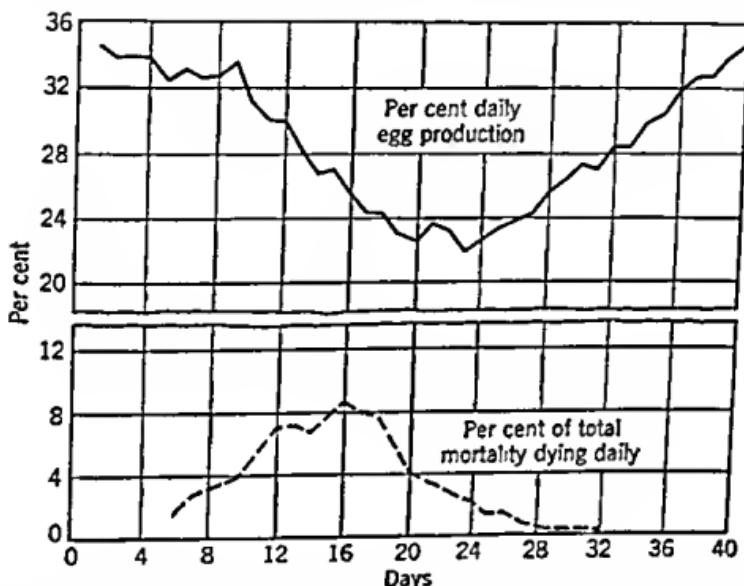


FIG. 77. Effect of infectious laryngotracheitis on livability and egg production. A composite grouping of data obtained in 25 outbreaks. (From Hinshaw, Jones, and Graybell, 1931.) Courtesy California Agr. Expt. Sta. and Poultry Science.

Vaccination is an effective means of preventing infectious laryngotracheitis. To insure maximum production and hatchability, hatcherymen operating in infected areas should insist that their breeder supply flocks be vaccinated before producing eggs.

Infectious Bronchitis

This is primarily a disease of young chicks, and for this reason can easily be introduced in a flock through the medium of a hatchery that operates a started-chick business. Extreme care should therefore, be taken by the hatchery operator to prevent accidental infection of his premises with this disease. Jungherr and Terrell of the University of Connecticut have reported in a

personal communication that they obtained evidence that infectious bronchitis immune bodies are transmitted through the egg. If the disease is introduced into the hatchery, depopulation of all chicks being brooded on the premises is advised. Thorough cleaning and disinfection of the premises should follow this procedure.

Infectious Coryza

This disease, caused by *Hemophilus gallinarum*, may also be transmitted in the hatchery if it becomes prevalent on the premises. It is not, so far as is known, ever transmitted through the egg. Since the disease may seriously affect breeding-flock operations, every effort should be made to prevent it from becoming established in the flock. Treatment with sulfonamides has been recommended, but only for temporary control, because these drugs do not permanently eliminate the causative organism from the infected flock. Complete segregation of all young stock from infected or carrier adult stock and depopulation for a period are methods recommended for the prevention of this disease (Beach, 1943).

Infectious coryza is one of the most costly of all poultry diseases, and no entirely satisfactory method of control has been found. Depopulation, followed by the cleaning and disinfection of the premises, and the purchase of day-old chicks as replacements are probably the most successful procedures. In areas where there are numerous poultry farms, however, the flock is constantly in danger of reinfection from near-by farms where there is infection, through air-borne infected dust, visitors from such places, exchange of equipment, and other mechanical means of transmission. Segregation of infected groups of birds on the same ranch is seldom successful, because of the many opportunities for carrying infection from the diseased flock to the healthy flock. No other disease needs further investigation more than this one, especially in its prevention and control phases.

RESPIRATORY DISEASES OF TURKEYS

Turkeys are resistant to the viruses of infectious laryngotracheitis and infectious bronchitis but can be infected with

Hemophilus gallinarum, the causative organism of infectious coryza. This disease, however, is seldom seen in turkeys.

The most common respiratory disease of turkeys is called infectious sinusitis (swell head). Often in this disease the air sacs are also involved, and these cases are usually called air-sac infections. The complete etiology of this group of diseases has not been determined. No evidence for considering infectious sinusitis an egg-borne disease has yet been presented but, like respiratory diseases of chickens, it may be transmitted in the hatchery.

Turkeys, like chickens, are not as efficient producers if infected with infectious coryza, and infected flocks should not be used for breeding purposes. Methods of control and prevention may be found in Biester and Devries (1943) and Barger and Card (1943). Reports on treatment are given by McNeil and Hinshaw (1946a; 1946b).

So little is known about the cause of the various respiratory diseases of turkeys that no definite recommendations can be made. There is little doubt that research work, which is badly needed on respiratory diseases of turkeys, will reveal several causative agents. Until these are known, research on control, prevention, and treatment is futile. Especially needed is intensive work on the group of diseases commonly called air-sac infections. In our own studies we have found that in addition to air-sac infection associated with sinusitis, the *Salmonella* group of organisms may cause similar lesions in the air sacs. Also, one of the manifestations of Newcastle disease is an inflammation of the air-sac membranes.

NEWCASTLE DISEASE (AVIAN PNEUMO- ENCEPHALITIS)

This disease, caused by a filterable virus, was first observed in the Dutch East Indies in 1926 by Kraneveld, according to Beau-dette (1943) and was later described in England by Doyle (1927) as Newcastle disease. Stover (1942) was the first investigator in the United States to show that a disease prevalent in California and described by Beach (1941) as a nervous-respiratory disease of chicks was caused by a filterable virus. Stover isolated the

Evidence that pneumoencephalitis *may* be transmitted through eggs from infected flocks, supporting similar evidence reported by others, has recently been obtained in three instances as follows:

- (1) The virus was isolated from the unabsorbed yolk of four-day-old chicks, hatched from eggs laid by an infected breeding flock when the egg yield was at the lowest point. The brood of chicks, including those from which the virus was isolated, appeared normal but pneumoencephalitis developed later and approximately 25 per cent died by the time they were three weeks old. No evidence of the infection appeared in two other lots of chicks from the same hatch which went to other farms.
- (2) The virus was isolated again from two dead embryos of the next succeeding hatch of eggs from the same flock. The embryos were dead on the fifteenth day of incubation. Five living embryos were also examined for virus but none was demonstrated.
- (3) The virus has also been isolated from the pooled contents of eight infertile eggs laid by a flock in which pneumoencephalitis was present. These eggs were incubated for seven days before they were examined for the virus.

Attempts to isolate the virus from developing embryos in eggs from three flocks completely recovered from pneumoencephalitis were unsuccessful.

During the year, pneumoencephalitis infection was detected in a very closely supervised and observed flock which had been and continues to be free from any symptoms of the disease.

In spite of these reports, millions of day-old chicks have been shipped from hatcheries located in areas where the disease is prevalent, and no subsequent cases of the disease have been reported that could definitely be traced to egg transmission. There are many examples, however, of outbreaks in chicks where the evidence indicated hatchery infection of the chicks after hatching. In these instances the infection has usually resulted from mechanical transmission in incubators and brooders, from started chicks, or from diseased flocks on the premises. There are a few examples in the literature that suggest infection of chicks en route from the hatchery to the purchaser (Jungherr and Terrell, 1946). There are numerous examples of transmission from one flock to another and even from one state to another through the importation of adult breeding birds that proved to be carriers. Beach (1942) isolated the virus from two adult hens 2 months after their visible symptoms had disappeared.

virus and reproduced the disease in chicks. Later this virus was reported to be immunologically identical with the virus of Newcastle disease by Beach (1944) and by Brandly, Moses, Jones and Jungherr (1946). Excellent reviews on this disease, as it has existed in other countries as well as in the United States, are given by Beaudette (1943) and by Brandly, Moses, Jones, and Jungherr (*op. cit.*).

In the United States the disease has caused the heaviest mortality in the brooding and the rearing stages. An outbreak in laying flocks has always been succeeded by a marked drop in egg production (Beach, 1942). Eggs laid during the outbreak of the disease are likely to be small, and the shells soft or rough and irregular. Lorenz and Newlon (1944) showed that 10 per cent of the eggs laid by a flock for 45 days after the disease had subsided failed to form normal air cells. Shell and albumen quality were also lowered. Although mortality in laying flocks is not usually high, the survivors are often subnormal. Thus the combined loss resulting from the disease through lowered egg production, decreased egg quality, increased mortality, and cull birds is of great economic importance to the grower.

Hatchery and egg transmission have not caused serious losses, but it is generally admitted that such transmission is possible. Here again, the started-chick business is more likely to be the source of hatchery transmission than is actual egg transmission. Brandly, Moses, and Jungherr (1946) reported that chicks hatched from eggs laid by recovered or immune hens were immune for 2 weeks to 6 weeks after hatching.

The fact that the virus of this disease has been isolated from eggs, newly hatched chicks, and dead embryos indicates that egg transmission is possible. At the first National Conference on Newcastle Disease (1946) Van Rockel reported the isolation of the virus from a fresh egg laid during an acute outbreak. Jungherr and Terrell (1946) reported the isolation of the virus from fresh hatching eggs produced immediately after cessation of an outbreak. In a release from the Department of Veterinary Science, University of California (1947) the following statement on egg transmission is made, concerning findings later reported by DeLay (1947):

Evidence that pneumoencephalitis *may* be transmitted through eggs from infected flocks, supporting similar evidence reported by others, has recently been obtained in three instances as follows:

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- (2) The virus was isolated again from two dead embryos of the next succeeding hatch of eggs from the same flock. The embryos were dead on the fifteenth day of incubation. Five living embryos were also examined for virus but none was demonstrated.
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Every effort should be made to prevent this disease from spreading. It is important that susceptible adult flocks be protected because of the deleterious effects of the disease on egg production and egg quality. Such protection is also important because of the possibility of transmission through the eggs of birds having the disease or recently recovered from it, and also because of the dangers of mechanical transmission from such flocks to the hatchery.

The following are selected recommendations from a detailed list prepared by the National Committee on Newcastle Disease (1946).

- (1) That it should be recognized that detection of infected flocks at the earliest possible moment is the first requisite for suppression of Newcastle disease, and that primary diagnoses should be made only by laboratory procedure.
- (2) That transportation of birds or carcasses for laboratory diagnosis should only be by private vehicle or carrier.
- (3) That vaccination with inactivated Newcastle disease virus vaccines may be advantageous where exposure to infection has occurred very recently or may be imminent or likely.
- (4) That no hatchery produce or offer for sale chicks or pouls originating in flocks showing evidence of active infection with or recently exposed to Newcastle disease.
- (5) That the danger of using old and dirty egg crates, fillers, and flats for the transportation of hatching eggs be recognized and guarded against by all practical means. It is further recommended that eggs be packed in a clean room other than the poultry house.
- (6) That all unnecessary visitors, all started chicks and pouls, and all persons handling other poultry be excluded from the hatchery. Hatcheries selling started chicks or pouls should segregate these birds from newly hatched poultry.
- (7) That all dead birds, offal, and hatchery waste be disposed of by rendering, incineration, chemical disinfection, or by deep burying.
- (8) That source flocks be inspected periodically for health.
- (9) That hands be thoroughly washed, and that all outer garments, footwear, instruments, and equipment used by chick sexers, cullers, blood testers, and others who must visit poultry premises be disinfected before leaving each premises.
- (10) That all crates and other equipment be thoroughly cleaned and disinfected with a saponified cresol solution permitted for official disinfection, before admission to the premises.
- (11) That feed bags be reused only after thorough cleaning.

- (12) That all crates and vehicles used for transporting poultry for slaughter be cleaned and disinfected after each use.
- (13) That cull poultry rejected by a processor not be returned to the farm or offered for sale to other processors.
- (14) That poultry offal and manure from all processing plants be disposed of in such a manner as to prevent the spread of Newcastle disease through these means and with retention of feed or fertilizer value of these materials. The manner of such disposal should be approved by the State live stock sanitary official.

It will be recognized that observing these recommendations will aid in preventing most of the diseases discussed in this chapter. Another recommendation that should be added is to avoid the purchase of started chicks or poultts and adult breeding stock for breeding-flock replacements.

AVIAN LEUKOSIS COMPLEX

Excellent reviews on this group of diseases which include the various forms of lymphomatosis are presented by Olson (1940) and Jungherr (1943). These references include a summary of the literature on the possibilities of egg and hatchery transmission. The data on egg transmission are limited but are supported by the more recent investigations of Waters and Priekett (1944) and Waters (1945). It is generally accepted that there are strain differences in resistance and that highly resistant strains within a breed can be developed by progeny-test selection. Waters maintains that it is possible to develop a flock that is free from the disease and that eggs from such a flock will hatch into disease-free chicks, which will remain disease free if kept properly segregated.

As stated by Hutt, Cole, Ball, Bruckner, and Ball (1944) there are two generally recognized methods of controlling this group of diseases. One of them is to breed strains that are resistant, and the other is to rear chicks in complete isolation from adult fowls during the growing season. The first method, demonstrated by Hutt, Cole, and Bruckner (1941) and Taylor, Lerner, DeOne, and Beach (1943), depends on the grower's ability to maintain the necessary exposure to the disease and to carry out the progeny testing on a long-term program. This plan is, however, a feasible one for the breeder-flock owner and furnishes a method by which resistant chicks can be supplied. DeOne

(1943) has demonstrated that the percentage of mortality from the disease is influenced by the level of exposure. He showed that even in highly resistant strains the incidence increased as the level of exposure increased. This fact may account for unexpected losses from the disease in chickens purchased from hatcheries that have highly resistant strains, as tested under a particular local environment, when they are exposed to a higher level of infection in a new environment. The possibility of multiple strains of the causative agent may also account for increased losses from the disease when chicks are shipped from one area to another. If more than one strain exists it would be possible for chicks from parent stock resistant to one strain to be very susceptible to a strain that may exist in another area.

Until more is known concerning the cause, or causes, and modes of infection of avian leukosis, a combination of the two methods outlined is the best recommendation for reducing to the minimum the mortality from this group of diseases. Every effort to develop resistant strains of fowl should be made by the breeder, so that resistant chicks can be produced. The buyer in turn should practice a segregation program to prevent undue chance of transmission from adults. Hutt *et al.* (1944) found that segregation for as short a period as 2 weeks following hatching will reduce to a large extent the mortality in later life in both resistant and susceptible stock. Segregation of young stock from all older birds, however, should be continued until maturity.

In a 1947 paper, Hutt and Cole reviewed the genetic control of lymphomatosis in the fowl and reported the results of 12 years' work on this problem at Cornell University. A method they suggested as feasible for developing resistant strains and maintaining flocks that are resistant is to provide cockerels from resistant flocks to head flocks that supply hatcheries, thus distributing the desirable genes.

This disease is generally considered the most costly of all poultry diseases. In spite of the enormous amount of research which has been done and is under way, there are still many problems to be solved before much hope of adequate control can be expected. The problem of whether the disease is transmitted through the egg must be settled; the causative agent or agents must be definitely determined; drugs for control need to be i-

vestigated; and the possibility of air-borne infection needs study. These are only a few of the problems involving leukosis which need to be investigated.

Increasing numbers of reports are being made on the occurrence of this group of diseases in turkeys, but the problem is not yet a serious one in the turkey industry.

OMPHALITIS (NAVEL INFECTION, MUSHY CHICK DISEASE)

This disease, characterized by edema of the breast muscles and by abdominal ascites is the result of the infection of the chick in the hatcher with one or more of a miscellaneous group of micro-organisms that are contaminants of the incubator. Entrance of the organisms into the body of the chick or poult is usually through the navel, and a failure of the navel to heal properly may predispose hatching chicks to the infection.

Volkmar (1929) was the first to report the presence of the disease in the United States. Brandy (1932) studied it more extensively and was the first to suggest formaldehyde fumigation as an aid in eliminating the infection from the hatchery. Williams and Daines (1942) determined that *Staphylococcus aureus* was the causative agent in an outbreak which they studied. They presented evidence that incubator operators suffering from *staphylococcus* infections may occasionally be responsible for the contamination of incubators. They also reported a case of staphylococcal dermatitis in a man who brooded turkeys from an infected hatchery. Glover (1944) reported that omphalitis may also be caused by *pullorum* disease.

The severity of the disease depends on the nature of the infection. In severe outbreaks, chicks will be found dead at the time of removal from the hatcher and/or when sorted for shipping. Sometimes the incubation period of the disease is prolonged, and losses do not start until a day or two after the chicks are delivered. Dead embryos and chicks decompose very rapidly, and one of the first suggestions of an infected incubator or hatcher is the odor coming from the machine.

The infection, if not caused by *S. pullorum*, is readily controlled by proper sanitation. Bittenbender (1940) and Insko, Steele, and

Hinton (1941) recommend the fumigation of incubators between hatches with two to three times the usual amount of formalin and potassium permanganate, as a preventive measure. For a more detailed discussion of fumigation see the chapter on Physical Conditions in Incubation (p. 209), and the section on formaldehyde in this chapter (p. 367).

Ascites from Other Causes

Ascites caused by an excess of sodium compounds including common salt (Scrivner, 1946) and by exposure to certain disinfectant fumes (Bullis and Van Roekel, 1944) are sometimes confused with omphalitis. Ascites (dropsy, watery belly, etc.) due to chemical poisoning is seen in young poult or chicks of 2 or 3 days to 2 weeks of age. Excess of salt in the ration and fumes from certain types of disinfectants used for spraying brooder floors are common causes of ascites. Excess of salt in the ration is usually accidental and may be due to improper feed mixes or to poor screening which allows lumps to get into the mix. The addition of salt to mashes that already contain it in the form of salted protein concentrates has also been responsible for a few outbreaks. Hatcherymen should warn customers of the bad effects of excess salt in starting mashes and of the danger of putting chicks into brooders too soon after spraying the floors with volatile disinfectants.

MISCELLANEOUS DISEASES

Many of the important diseases of fowls are definitely *not* egg borne or hatchery transmitted. These include most of the protozoan diseases, such as coccidiosis, leucocytozoon infection, black-head, and hexamitiasis; and the fungus diseases which include aspergillosis or brooder pneumonia, favus, and moniliasis. The following virus and bacterial diseases are, as far as is known, never egg borne and seldom if ever hatchery transmitted: fowl pox, crypsiplas, botulism, and fowl cholera. The internal parasites (roundworms, tapeworms, etc.) and external parasites (lice, mites, ticks, etc.) may also be thus described. Although these diseases are not transmission problems of the hatchery, efforts should be made to prevent them from causing losses in breeding

flocks. All of them can cause economic losses in the susceptible flock and some, like fowl cholera and fowl pox, may greatly impair the efficiency of breeding flocks.

Avian Encephalomyelitis (Epidemic Tremors)

This disease of young chicks which is caused by a filterable virus may be egg borne and hatchery transmitted according to Van Roekel, Bullis, and Clarke (1938) and Van Roekel, Bullis, Flint, and Clarke (1943). Unpublished research work done by Van Roekel and his colleagues since 1943, reported in a personal communication, has substantiated the previous results, and these investigators consider that the disease should definitely be added to the list of egg-borne infections. They advise flock owners to avoid using for breeding purposes any flock that has suffered from the disease.

INCUBATOR AND HATCHERY SANITATION

Two classes of disease have to be taken into account in any program for prevention of disease in the hatchery. The first includes those diseases that are definitely egg transmitted. The second includes diseases that are transmitted, after the chicks or poultts are hatched, by contact with disease-producing micro-organisms introduced from sources other than the egg. Examples of the first group are pullorum disease and other salmonelloses. Examples of the second group are omphalitis, Newcastle disease, and the respiratory diseases.

Prevention of the first group is best effected by using eggs from disease-free supply flocks. Means for accomplishing this are discussed more fully in the sections on the specific diseases. Prevention of the second group depends largely on the standards of sanitation maintained, especially within the hatchery. If a program of prevention is to be successful, every effort must be made to avoid the keeping of any birds (including started poultts and chicks) on the hatchery premises.

It is also important in the prevention program that hatcheries employ no labor that has contact with other poultry or hatcheries. Such help may act as mechanical carriers of diseases from other birds to poultts or chicks hatching or being handled in the hatch-

ery. An example of man-transmitted omphalitis is given by Williams and Daines (1942). Chick sexers are potential carriers of disease from one hatchery to another. One outbreak of pullorum disease investigated by the writer followed the sexing of poultts by a person who had gone from one hatchery to another without changing his clothing or making any effort to cleanse and disinfect his hands or equipment. Operators should be required to put on clean clothing and wash thoroughly at each hatchery.

Other examples of mechanical carriers that may spread disease in the hatchery are mice, rats, flies, and wild birds. Hatchery equipment is another possible carrier of infection and should be used only for the hatchery; if it is used elsewhere, it should be thoroughly cleaned and disinfected before it is again used in the hatchery. Only new chick and poult boxes should be used, and, if an emergency requires that old boxes be used again, they should be fumigated before such re-use. Empty egg cases constitute another possible source of infection. There are no completely satisfactory methods for disinfecting such cases although formaldehyde fumigation is sometimes used. A fumigation room is necessary for this method of disease control. It is very important that egg cases be labeled and always returned to the farm from which they came.

If hatcheries are made proof against rodents, as well as flies, the transmission of disease will be reduced. Proofing to prevent entry of rats and mice is discussed in detail by Silver, Croueh, and Betts (1942) and by Storer (1942). The use of DDT at regular intervals will help to control flies and other insects. This product may be toxic for birds and should always be applied according to the manufacturer's directions.

Disinfection

Disinfection is important in reducing the chances for the transmission of certain diseases within the hatchery, and this subject has been discussed under the headings of the specific diseases. A summary of some of the essentials for proper disinfection are given below, together with brief discussions of some of the common disinfectants.

It is essential in any sanitary program to clean the hatchery before disinfecting. It is also imperative to include every room

and piecee of equipment and not the incubators alone in such a program. The following steps are suggested for cleaning and disinfecting:

1. Settle all dust wherever possible by a light spraying with the disinfectant. This procedure avoids undue scattering of germs by dust.



FIG. 78. An example of a high-pressure steam cleaning machine in operation in a hatchery. *Courtesy S. E. Hall Co. and Poehlmann Hatchery.*

2. Remove all refuse and haul immediately to the disposal area (see Disposal of . . . Hatchery Refuse, p. 372).

3. Remove all movable equipment to a cleaning platform which should be a part of every hatchery.

4. Thoroughly spray every part of the hatchery with a standard disinfectant. If a high-pressure steam cleaner is available, disinfection and cleaning may be done in a single operation (see fig. 78).

5. Follow the procedure recommended by the manufacturer for cleaning and disinfecting the incubator.

Much is now being written about air transmission of disease, and it is recognized that such transmission is important for many diseases. In hatcheries, the air transmission of pullorum disease

and omphalitis has long been recognized. It was in an attempt to prevent this form of transmission that Coon (1928) and Bushnell, Payne, and Coon (1929) did the first research work on formaldehyde fumigation as a means of disinfecting forced-draft incubators. This method of disinfection is now in general use in the hatchery industry and is described in chapter 6. Ultra-violet lights and glycol vapors have been exploited with varying degrees of success as means of air disinfection in hospitals, and in army and navy barracks. Their use has not been so successful for air disinfection of hatcheries because of the protection afforded the organisms by the chick down (DeOme, 1946).

Disinfectants

The number of chemicals sold as disinfectants is so great that the prospective buyer is often bewildered. Some are worthless; others are excellent disinfectants but have undesirable characteristics. An ideal disinfectant should be: (1) low in cost per unit of disinfecting value, (2) readily soluble in hard water, (3) relatively safe for man and animals, (4) efficiently deodorant, (5) readily available, (6) harmless to utensils and fabrics, (7) stable when exposed to air, (8) free or nearly free from objectionable and lingering odor, and (9) effective for a large variety of germs. Obviously, no one chemical will have all these properties, but the list will serve as a guide.

Many disinfectants of similar composition are sold under different trade names. The phenol coefficient gives a fair estimate of their relative effectiveness. Before a product is purchased under an unfamiliar trade name, types and values should be compared with a well-known product. The directions for dilution given by the manufacturer should be followed in making up a disinfectant for use. These directions are usually based on the concentration of the product; by comparing the dilution factor of two disinfectants having other properties equal, one can determine the relative cost of the two.

Below are listed a few of the common disinfectants used in hatcheries:

(1) Lye. Lye is an excellent cleansing agent, valuable in any disinfecting program. A 2 per cent solution of sodium hydroxide (soda lye) is a good disinfectant for many of the germs causing

disease. Because of insufficient data on its power to kill some of the common poultry-disease germs, however, it should be used



FIG. 79A. Fumigation of forced-draft incubators with formalin. Cheese cloth method. The formalin-saturated cheese cloth is "ballooned" by the air currents of the fan which quickly disseminates the formaldehyde gas throughout the machine. (From Graham and Michael, 1937) Courtesy Illinois Agr. Expt. Sta.

primarily as a preliminary cleaning agent. As it is a severe caustic, it should be handled with care.

(2) FORMALDEHYDE. Formaldehyde is a gas, sold commercially in a 40 per cent solution in water, under the name of formalin. As a spray it is used in a 10 per cent solution of formalin (that

is, a 4 per cent solution of formaldehyde). Though a powerful disinfectant, it has many disadvantages, especially its volatility, penetrating odor, caustic action, and tendency to harden the skin—properties which make it disagreeable to apply. Its chief advantages are that: (1) it can be used as a gas or vapor for fumigation of incubators or small rooms; (2) it is relatively non-



Fig. 79B. The potassium permanganate-formalin method of disseminating the gas. (From Graham and Michael, 1937.) Courtesy Illinois Agr. Expt. Sta.

toxic to animals and fowls; (3) it is an efficient disinfectant in the presence of organic matter; and (4) it does not injure utensils and spraying equipment with which it comes in contact.

Fumigation of incubators and incubator rooms is a common practice of hatcherymen (fig. 79A and B). Most manufacturers have recommendations for their type of incubator, and, when possible, these directions should be followed. When fumigating a room or an incubator, the space must be air tight, and the room temperature and humidity as high as possible (see Precautions Necessary in Fumigation, p. 234, for methods of using formaldehyde).

(3) CAESOL. Cresol is a thick yellow or brown liquid, miscible with water but only slightly soluble. It forms the basis for many of the best commercial brands of disinfectants, made by combining cresol with a soap base.

Compound solution of cresol (*liquor cresolis compositus*, U.S.P.) is the most refined of the saponified cresol solutions. These are more effective and less toxic than phenol, can be greatly diluted, are reasonably priced, and are fairly stable in the presence of organic matter; but they have the disadvantage of being soapy and of having the odor characteristic of the cresols. They can be recommended for general use on the farm.

(4) CHLORINE GAS. Chlorine gas is the basis of the disinfectants known as hypochlorites. The numerous brands of these products offered for sale vary in their value as disinfectants according to their chlorine stability and their ability to liberate chlorine gas. They should contain at least 2.6 per cent by weight of available chlorine, the active disinfecting element of such products. These solutions are highly efficient if used according to directions. Their chief disadvantage lies in the instability of the chlorine when exposed to air or organic matter. They are also quite expensive. Their principal use is for disinfecting limited areas such as incubators, small brooders, and water and feed containers.

(5) CHLORINATED LIME. Chlorinated lime, known as bleaching powder, is prepared by saturating slaked lime with chlorine gas. It should contain 30 to 35 per cent of available chlorine. The Bureau of Animal Industry of the U. S. Department of Agriculture recognizes chlorinated lime for purposes of official disinfection when it contains at least 30 per cent available chlorine and when it is used in proportions of 1 pound to 3 gallons of water. Products containing less available chlorine should be used in more concentrated solutions. The final dilution should contain approximately 1.2 per cent of available chlorine by weight. Fresh solutions must be prepared daily. All products containing chlorine must be handled with care, since free chlorine is destructive to fabrics, leather, and metal.

(6) QUICKLIME (UNSLAKED LIME, CALCIUM OXIDE). The action of quicklime depends on the liberation of heat and oxygen when the chemical comes in contact with water. On the poultry ranch

its use is limited to small yard areas that are damp and cannot be exposed to the sun, to the disinfection of drains and fecal matter, and to whitewashes. Adding chlorinated lime to quicklime at the rate of 1 pound to 40 gallons of wash increases its disinfecting value in whitewashes. Quicklime may be used to aid decomposition of carcasses in disposal pits.

(7) SODIUM ORTHOPHENYLPHENATE. This substance has recently been recommended as a general disinfectant. It has no objectionable odor, is relatively nontoxic, highly effective against most disease germs, and readily soluble in water. It may be purchased in the form of grayish, brownish, or white powder or flakes, which must be kept in a closed container to prevent deterioration. It is now sold under several trade names, which are included on the U.S.D.A. Bureau of Animal Industry list of permitted disinfectants. It gives best results when it is applied hot. According to trials made by the United States Department of Agriculture, it is effective for control of mites and lice.

(8) QUATERNARY AMMONIUM COMPOUNDS. Much publicity is being given to quaternary ammonium salts as disinfectants. There are a number of these products now on the market which are generally considered efficient, if used according to directions. They are water clear, odorless, nonirritating to the skin, and have a marked detergent (cleansing) action. They are recommended especially for disinfecting eggs and for general use around the hatchery. It is important to remember that these products cannot be used in soapy solutions.

(9) GLYCOL COMPOUNDS. The germicidal action of glycols on air-suspended bacteria and viruses was first demonstrated by Robertson, Bigg, Miller, and Baker (1941). Attempts to adapt them for disinfecting the air in incubators have been made by several investigators (DeOme, 1944, 1946; and Gwatkin, 1947). Direct application to hatchery use was reported by Gwatkin, who concluded that propylene or triethylene glycols have little value as incubator disinfectants.

Disinfection against Newcastle Disease

Tilley and Anderson (1947) studied the germicidal action of several disinfectants on the virus of Newcastle disease. They found that the following were effective against the virus in the

percentage solutions given: sodium hydroxide, 2 per cent; sodium orthophenylphenate, 1 per cent; *liquor cresolis saponatus* U.S.P., 1 per cent; and quaternary ammonium compounds (2 tested), 0.1 per cent. Formalin in 4 per cent solution failed to inactivate the virus after 30 minutes but killed it after 60 minutes. Calcium hypochlorite solution of a low alkalinity (*pH* 8.25) was effective in a solution of 400 parts per million, whereas hypochlorite of high alkalinity (*pH* 11.4) in the same dilution failed to inactivate the virus. Under the conditions of the tests, the following disinfectants were ineffective against Newcastle disease virus: isopropyl alcohol, 50 per cent; ethylene glycol, 100 per cent; and sodium carbonate, 4 per cent.

The results given above are important to the hatcheryman since investigations on the control of Newcastle disease suggest the disinfection of eggs at the farm, or immediately on arrival at the hatchery, as one means of preventing the spread of the disease. If, for example, formalin fumigation is to be used for this purpose, it should be remembered that short-time exposures may not be effective. If other disinfectants are used the eggs must be dipped in the solutions, and unfortunately not too much is known about the effects of such a procedure on hatchability.

Boyd (1928) concluded that various commercial disinfectants which he tried for shell disinfection (Sterilac, sodium hypochlorite, iodine suspensoid, and chlorinated lime) affected hatchability slightly and that dead embryos were more numerous in the disinfected lots than in the nondisinfected lots. Thorp (1930) tested 7 disinfectants (95 per cent alcohol, 1.0 per cent phenol, 5.0 per cent phenol, 1-5000 mercuric bichloride, 1-10,000 mercuric bichloride, 1-1000 Sterilac, and 1.0 per cent Lugol's solution of iodine) on eggs and observed no harmful effects on hatchability. Olsen and McNally (1947), testing 2.0 per cent solution of sodium hydroxide, 1.0 per cent solution of sodium orthophenylphenate, and 0.1 per cent solution of a quaternary ammonium compound, compared the hatchability of disinfected and nondisinfected eggs and found no differences.

In a release from the United States Department of Agriculture (1947), one of the quaternary ammonium compounds or a chlorine solution such as the calcium hypochlorite described above is suggested for dipping hatching eggs before setting to kill Newcastle

disease virus. Sodium orthophenylphenate in a 1.0 per cent solution has also been suggested for this purpose. The need for research on the effect of such disinfectants on hatchability is evident from the lack of accurate information available.

DISPOSAL OF DEAD CARCASSES, EGGS, AND OTHER HATCHERY REFUSE

Methods of disposal of hatchery waste include incineration and burying. Incineration, *if done properly*, is a means of reducing the chances of spreading disease from such waste. Matter to be incinerated should be put into the fire pit immediately and not

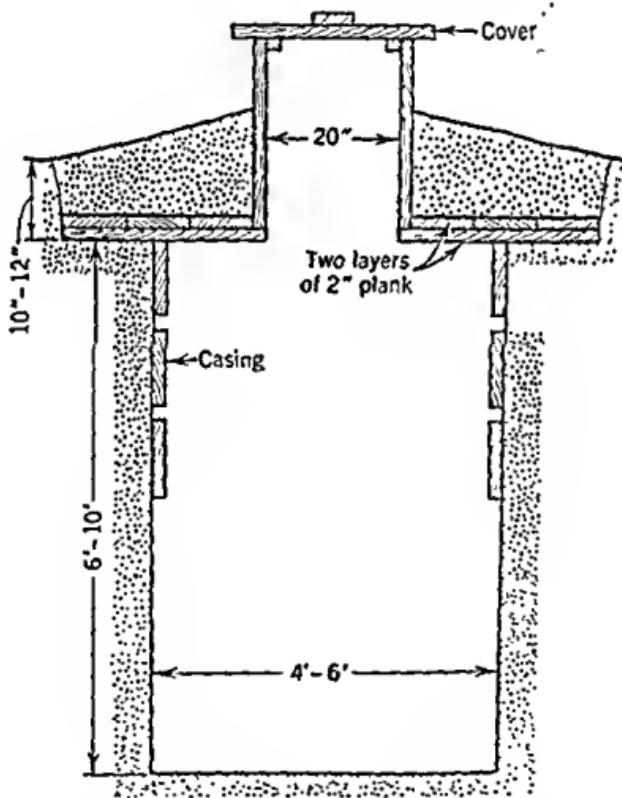


FIG. 80. Cross-section drawing of a poultry disposal pit, which can be used also for disposal of hatchery wastes. Such a pit should be located on ground that drains away from the farm water supply. Courtesy California Agr. Expt. Sta.

left outside where it is exposed to flies. Complete burning is essential.

Disposal pits like that illustrated in figure 80 are widely used. Such pits are easily constructed and are cheap and efficient. The roof and especially the "manhole" covering must be air tight to prevent the escape of odors which might attract flies. Quicklime may be used to hasten decomposition, as suggested for manure pits by Yushok and Bear (1944), but it is not necessary. Periodic spraying of the roof of the pit with 5.0 per cent DDT is suggested to kill the few flies that may be attracted. *Open pits are not recommended.*

Some hatcherymen dispose of infertile eggs, dead chicks, and dead embryos to hog raisers. This procedure may be a convenient and profitable one but, unless the refuse is thoroughly cooked before it is fed, the hog farm becomes another source disseminating such diseases as pullorum disease and other salmonelloses.

The truck that hauls hatchery refuse should be cleaned and disinfected in some way. Steam cleaning by a high-pressure steam cleaning device like the one illustrated in figure 78 is recommended. Liberal use of DDT on the truck body and around the loading area is also advised.

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CHAPTER 9

Education and Research in Fertility and Hatchability

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The foregoing chapters have detailed our present scientific knowledge of fertility and hatchability. The authors have indicated some of the problems that must be solved before the poultry breeder and the hatcheryman can attain better results. This chapter will be devoted to an examination of the various ways in which these problems may be studied and our fund of both fundamental and practical information thereby increased.

LIMITATIONS OF CURRENT RESEARCH

Lack of Trained Personnel

Perhaps the most important factor limiting the present amount of research on hatchery problems is the lack of suitably trained personnel. Few trained embryologists have interested themselves in studies applicable to the practical phases of the poultry industry. On the other hand, few students majoring in poultry have elected to specialize in the sciences that are necessary for an understanding of embryonic development. A graduate curriculum in applied experimental embryology could be developed with the high scientific standards that exist today in the fields of animal nutrition, genetics, animal physiology, and animal pathology.

Lack of Specific Projects

A second factor limiting the effectiveness of research on hatchability has been the fact that the projects in operation usually have been designed to solve other problems; the results derived, therefore, that are of interest to the hatcherymen have been a by-product of the studies. As a consequence of this emphasis, the study of embryological problems has often been deferred, pending the solution of other phases of the research program. For example, the importance of studies on the nutritional requirements of the embryo was recognized much later by biochemists than was the need for determining the requirements for posthatching growth or for egg production. Similarly, the geneticist has been more concerned about the inheritance of egg production and morphological characters of the bird than about either fertility or hatchability. Although pathologists may be credited with early recognition of the role that the incubator may play in the spread of poultry diseases, the proof of egg transmission of diseases produced by unidentified agents still remains in urgent need of further investigation. More projects should be specifically designed to apply directly to the problems of the hatcheryman.

Complex Nature of Problems

A third limiting factor is that the solution of a hatchery difficulty may prove to lie in any one of many different scientific

fields. For example, a condition suspected to be genetic in origin may eventually prove to have been caused by a nutritional deficiency. In this instance a geneticist would find the solution of the problem to be outside his field of training. Other hatchery problems may result from the interaction of several variables. When the problem seems to be complex, the leadership of a project by scientists trained in several different disciplines in co-operation with a poultryman capable of handling embryological work would be superior to an investigation conducted by only one person. Aid from the following scientific disciplines may be considered for such co-operative projects: genetics, physiology, endocrinology, biochemistry, nutrition, bacteriology, pathology, biophysics, and engineering.

Inadequate Knowledge of Literature

A fourth limitation has been that few attempts have heretofore been made to collect and collate information on fertility and hatchability in poultry. The most adequate available summaries were developed by Lippincott (1927) for two chapters in a general textbook on poultry production and by Landauer (1937) in a bulletin of the Storrs Agricultural Experiment Station. The preceding chapters of this book will provide to poultry instructors and investigators an essential knowledge of the extensive experimental work that has been performed and the source of scientific reports of research upon which practical and theoretical hatchery methods are based. Recognition of the possibilities of and the need for further research on subjects covered in the previous chapters should lead to the training of the necessary personnel and the organization of research projects for work on the specific problems of the hatchery business. College and experiment-station administrators, as well as poultry scientists and poultrymen, must appreciate the fact that research on fertility and hatchability constitutes a valid field for scientists.

Lack of Interest from Industry

A final necessity for an active research program in an applied field is appreciative and enthusiastic support from the members of the industry benefited. There has been a general apathy on the part of hatcherymen toward investigations on fertility and hatch-

ability. Today a rapid increase in volume of research conducted by industrial organizations and the allocation of grants from industrial firms or trade associations to colleges, experiment stations, and research institutes make possible a greatly enlarged scope for embryological studies on the problems of the poultry breeder and the hatcheryman. These studies can be further promoted by active interest and financial support from the national and state breeder and hatchery associations.

RESEARCH AND INSTRUCTION DEALING WITH AVIAN EGGS AND EMBRYOS

Research Institutions

All members of the poultry industry should appreciate the fact that valuable information is often obtained from experiments originally designed only to increase our knowledge of some phase of embryonic development. These studies are frequently carried out in departments or institutions having no relation to agriculture or to the practical applications of the embryology of domestic animals. In fact, modern methods of artificial incubation are based on scientific findings rather than on the craftsmanship employed for centuries in the hatcheries of Egypt and China (Romanoff, 1936).

Since the days of the Greek naturalists who are credited with the founding of biological sciences, poultry eggs have been favored material for studies of development. Hutt (1933) has discussed the importance of such studies to current methods of biological research, and Needham (1931) has given an excellent review of the historical development of the chemical phases of embryology.

Our industry has benefited not only from these extensive studies of the development of avian embryos but also from research conducted with other animals, which has sometimes discovered principles that later have been applied to poultry. An instance of this, the full importance of which may not yet be known, is the concept of organizers, which was developed from studies on amphibians and was later investigated by Waddington (1933a, b; 1934) in chick embryos. Organizers are presumed to be chemical substances developed in embryonic cells or tissues capable of inducing the differentiation of surrounding tissues into

specific morphological structures (see Needham, 1942). Further studies on the nature of organizers in avian embryos and their role in both normal and aberrant types of embryonic development should be undertaken to determine whether the genesis of some common forms of monsters and other nonviable embryos may thus be explained on a chemical basis.

Departments of zoology almost always have staff members trained in embryology and frequently support research studies in this subject. Medical and veterinary schools, departments of biochemistry, biophysics, and physiology, and various kinds of research institutes often conduct work on chick embryos. The results of such studies frequently can be applied in poultry practices. The industry should neither ignore the facts so discovered nor fail to encourage experimental work which may increase our basic knowledge of the embryology of the various species of poultry.

The major interest in research on the development of chicks and poult, however, will be found in the poultry husbandry departments of colleges of agriculture and state experiment stations which have the direct responsibility for instruction and research in the subjects of nutrition, genetics, and incubation of poultry. Supplementing the state institutions, and also functioning in research, are the federal experiment stations and substations. A reasonable doubt may be expressed as to whether the volume of work on fertility and hatchability in such colleges and stations is presently commensurate with the economic importance of the breeding and hatchery phases of the poultry industry.

Industrial research applied to poultry or conducted by manufacturers of incubators or other hatchery equipment has not yet developed to the point where the results obtained have greatly increased the general public knowledge. Privately conducted research has probably been responsible for the major improvements in incubator design and performance, but the data on which the improvements have been based have seldom been published.

Table 45 is offered as an attempt at a representative listing of some of the laboratories and institutions that should be recognized as possible places for research on the composition of avian eggs, their fertility, and their embryonic development.

TABLE 45

A REPRESENTATIVE LIST OF AGENCIES UTILIZING AVIAN EGGS OR EMBRYOS
IN RESEARCH PROGRAMS*I. Federal*

Department of Agriculture	Bureau of Animal Industry Bureau of Human Nutrition and Home Economics Bureau of Agricultural and Industrial Chemistry Bureau of Dairy Industry Fish and Wildlife Service Bureau of Medicine and Surgery
Department of the Interior	
Department of the Navy	
Department of the Army	{ Quartermaster Corps Medical Corps
Department of the Air Force	Aero-Medical Laboratory
Federal Security Agency	Public Health Service National Institute of Health National Cancer Institute
Atomic Energy Commission	Food and Drug Administration Research Division (Programs in Medicine and Biology)
Veterans Administration	Medical Research Division

II. State

Department of Health	Public Health Laboratories
Department of Agriculture	Bureau of Livestock Sanitary Control
Conservation Commission	{ Fish and Game Research Division Game Farms

III. Universities and Colleges

College of Agriculture

Agricultural Experiment Station

TABLE 45 (*Continued*)A REPRESENTATIVE LIST OF AGENCIES UTILIZING AVIAN EGGS OR EMBRYOS
IN RESEARCH PROGRAMS

College of Veterinary Science	Departments of Physiology, Anatomy and Histology, Microbiology, Pathology, Clinical Diagnosis, Pharmacology, Immunology
College of Medicine	Departments of Physiology, Biochemistry, Medical Physics, Anatomy and Histology, Microbiology, Pharmacology, Pathology, Immunology, Public Health
College of Letters (Arts) and Science	Departments of Zoology, Bacteriology, Physiology, Biochemistry, Biophysics

IV. *Research Institutes*

Public or Semi-Public Organizations for Research	Fields of: Nutrition, Experimental Biology, Medical Research, Animal Research
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V. *Industrial Organizations*

Research Departments	Manufacturers of Feeds and Feedstuffs, Medical and Veterinary Supplies, Chemicals and Pharmaceuticals, Incubators and Poultry Supplies; Poultry Breeding Farms, Hatcheries
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Collegiate Courses in Incubation

Instruction in incubation, as given in many agricultural colleges, is characterized by an insufficient number of credit-hours required and by a failure to present the basic information necessary to a clear understanding of the problems involved (Romanoff, 1938). Too frequently the courses deal only with the mechanical phases of the handling of hatching eggs and of operation of the incubator. Although important, an understanding of these phases alone is insufficient to prepare a student to meet many hatchery problems that arise constantly in the poultry industry.

Courses in embryology and organic chemistry or biochemistry should be required as prerequisites for an incubation course. If

the student has also taken work in physiology and genetics, he will be better prepared to understand the relation of these subjects to embryonic development. It follows from these recommendations that the incubation course should be restricted to members of the junior and the senior classes and to graduate students.

Instruction in incubation work can be organized in several ways, depending upon the major interests of the students enrolled. Romanoff has proposed a lecture and laboratory program of subject matter (table 46) which combines a general survey of

TABLE 46

SUGGESTIONS FOR SUBJECT MATTER OF LECTURES AND LABORATORY
EXERCISES FOR A SPECIALIZED SEMESTER COURSE IN
INCUBATION
(Romanoff, 1938)

Lectures (1 or 2 credit-hours)

Topics	Statement of contents
Hatching industry Principles and practice Natural incubation Biology of an egg	State and national scope of hatching industry Major problems of artificial incubation Physiological behavior of a sitting hen Formation, structure, and chemical composition of the egg
Production of hatchable eggs Selection of hatchable eggs Care of hatching eggs Embryonic development	Factors affecting fertility and hatchability of eggs Inheritance and physical characters of hatchable eggs Handling, care, and shipment of hatching eggs Structural development and chemical metabolism of the embryo
Environmental requirements History of artificial incubation Types of incubators	Optimum conditions and effect of adverse conditions Evolution in artificial hatching up to the present day Construction of incubators, incubator equipment, and incubator rooms

TABLE 46 (*Continued*)

SUGGESTIONS FOR SUBJECT MATTER OF LECTURES AND LABORATORY EXERCISES FOR A SPECIALIZED SEMESTER COURSE IN
 INCUBATION
 (Romanoff, 1938)

Incubator operation	Handling and operation of various types of incubators
Handling of a hatch	Care of incubated eggs and removal of hatch
Incubator sanitation	Disinfection, fumigation, and blood testing
Sexing and grading of chicks	Sexing, score-card judging, and factors affecting quality of chicks
Hatchery management	Commercial hatchery and its operation
Economics of incubation	Factors determining the economy of artificial hatching
National improvement plan	New trends in education and research

Laboratory exercises (1 credit-hour)

- Study of the gross anatomy and reproductive system of a hen (with demonstration of the reproductive system of a cock).
- Detailed study of morphological structure of the fresh egg (preferably of several species of birds).
- Observation of living embryos at various stages of incubation (through a hole in the shell and in the dissected egg).
- Comparative study of the construction and mechanical features of various types of incubators (from catalogues).
- Practical operation of different types of incubators with special reference to air movement and control of temperature, humidity, and air ventilation.
- Study of methods of selection and preparation of eggs for hatching.
- Care of eggs during incubation, methods of testing and calculating fertility and hatchability.
- Taking off the hatch, including leg and wing banding and pedigreeing of chicks.
- Detailed study of the gross anatomy of newly hatched chicks.
- Methods of culling, grading, judging, and sexing of chicks.
- Boxing and shipping of chicks and calculating costs of mailing and shipping to various points of the country.
- Demonstration of blood testing of chicks, disinfection and fumigation of incubators, and hygienic upkeep of incubator room.
- Statistical study of the costs involved in the production of chicks by artificial methods.
- Trip to a commercial hatchery for detailed study of equipment, methods of operation, handling of started chicks, and general problems.

the hatching industry with fundamental information on embryonic development and practical methods of hatchery operations. This program differs in a number of points from a course in experimental incubation which the writer has taught to major students and graduates (table 47). Although a general basic outline

TABLE 47

GENERAL PLAN OF A COURSE IN EXPERIMENTAL INCUBATION
(Prerequisite courses: embryology and organic chemistry)

Lectures (2 semester credit-hours)

1. Review of the formation of the gametes and of morphological development of the chick through the fourth day of incubation, followed by a detailed day-to-day developmental history of the embryo from the fifth day to hatching. (5 weeks.)
2. Subject matter as generally treated in the eight preceding chapters of this book. (5 weeks.)
3. The chemical and physiological ontogeny of the avian embryo. (4 weeks.)

Laboratory (1 semester credit-hour) given by appointment in a period of 26 consecutive days

1. Examination of daily stages of normal chick development from the uninoculated egg to hatching produced by the student in incubators under control of the class. This work involves dissections, microscopic and macroscopic studies, and tests for functional activity of organs. (75 per cent of the laboratory time.)
2. Examination of infertile eggs and dead embryos; experimental demonstrations of abnormal development produced by improper physical conditions, nutritional deficiencies, lethal genes, etc.; measurement of changes in unfed chicks for 72 hours after hatching. (25 per cent of the laboratory time.)

is desirable, modifications should be made in the subject matter to meet the individual needs of students having various specific reasons for taking the course.

Most embryologists lose interest in the development of avian embryos after the major organ systems have been formed and

terminate their instruction with the 96-hour embryo. A detailed, day-by-day account of the development of the chick or poult embryo to the end of the incubation period should be given in an incubation course, since the greater part of the mortality of avian embryos occurs after the fourth day (fig. 3, p. 16 in chap. 1). The normal state of development of the embryo and its relation to the fetal membranes and the food supply in the egg should be determined for each day of incubation. The ability to detect abnormalities representing lethal effects is dependent upon this knowledge of the normal course of development. The subject matter covered in the preceding chapters also should aid in the diagnosis of conditions related to infertility or the development of abnormal embryos.

The course should develop an understanding of the succession of organs participating in respiration, food assimilation, excretion and the disposal of waste products, and other vital processes of embryonic development. Although many phases of the normal functioning of embryonic organs are not clearly understood, in some types of lethality malfunctioning of certain organs is detectable, and the causes of such malfunctions are known. The practical application of this part of an incubation course lies in the diagnosis of causes of failure in hatchability observed in the hatchery. A routine examination of dead embryos should be undertaken in hatcheries when hatchability falls to an unsatisfactory level and at all times on breeding farms for the pedigree breeding flock. Lethal genes, some nutritional deficiencies, improper preincubation care of hatching eggs, faulty incubator operation, and disease can frequently be detected by abnormalities of the embryo or by the condition of the contents of the unhatched egg.

The college course in incubation should serve both as a terminal course for the student who is interested mainly in a general poultry training and as a preparatory course for the student intending to specialize in hatchery operations or research in hatchery problems.

Graduate Study Programs

As can be readily inferred from the preceding discussion, the instructor or investigator dealing with problems of the hatchery

business must have training or experience in a number of scientific fields. The graduate programs in most institutions do not offer a field of study designed specifically to train the developing scientist for the types of work that are involved in a research problem in incubation. The common graduate fields of zoology, genetics, and biochemistry come closest to furnishing the desired scientific background, but training in applied vertebrate embryology would permit a superior degree of specialization for a staff member in poultry husbandry responsible for incubation work. The writer has attempted in table 48 to detail the constitution of

TABLE 48

PLAN FOR A GRADUATE FIELD OF APPLIED VERTEBRATE EMBRYOLOGY

A field for students who have completed undergraduate majors in the animal sciences, biochemistry, biophysics, genetics, physiology, medical sciences, wildlife conservation, or zoology and are candidates for the degree of Doctor of Philosophy

Subjects serving as a general basis for examinations in this field

Foreign languages: preferably German and French

Zoology: advanced or experimental vertebrate embryology; cytology; microscopical technique

Anatomy: histology

Genetics: fundamental principles; animal, poultry, or human genetics

Biometry: elementary statistics

Biochemistry: animal biochemistry, animal or human nutrition

Physiology: mammalian or avian physiology

Biology: a general course in the biology of the species used as an experimental animal (for example: biology of man, farm animals, or poultry; ichthyology)

Optional subjects (not recommended for all students in the field, but desirable in certain programs)

Comparative anatomy, animal or human pathology, enzymology, endocrinology, experimental incubation, microbiology.

such a proposed program of graduate study for students who may desire training in the applications of veterinary or human embryology to medical practices, as well as in applied avian embryology. This general field might also serve to train scientists for research on problems related to fish hatcheries and the conservation of fish and game.

It is unreasonable to expect the experimenter in hatchery problems to have had intensive training in all the fields in which incubation research may fall. Certain subjects, however, are basic to most of the problems that may arise; in the opinion of the writer these are embryology, genetics, physiology, and biochemistry. A general knowledge of biophysics, anatomy or histology, bacteriology, pathology, and nutrition also would be helpful. When a problem proves complex, a group of representatives from a number of different scientific fields should be formed as a more effective staff for handling the research project. Project administrators should favor projects conducted by such groups of scientists and the formation of research committees to serve in advisory capacities. Thus the experience gained from many different scientific fields would be combined to bear upon the problem. Research in fertility and hatchability is particularly favorable for the operation of research groups.

FERTILITY AND HATCHABILITY GOALS

What should be the aim of efforts to improve the efficiency of hatchery operational methods? It is commonly recognized today in the poultry industry that a successful breeding and hatchery business cannot usually be sustained through both good and bad economic periods if less than 75 per cent of the eggs incubated are hatched. This percentage represents at least a 10 per cent increase over the hatchability levels considered satisfactory twenty years ago. Although the hatchability of the fertile eggs set has increased rather uniformly throughout the United States since 1930, in a disturbing number of breeding flocks the average levels of fertility have apparently decreased. Breeders of turkeys, in particular, have been concerned about the problem of maintaining satisfactory levels of fertility, and some chicken breeders also have had similar difficulty.

The poultry industry is probably justified in considering 95 per cent fertility and 90 per cent hatchability of fertile eggs as attainable goals. Evidence is available from some breeding flocks and hatcheries that even higher reproductive standards are occasionally obtained, but the means for producing such results consistently are not known. Eventually it should even be possible

to obtain averages for the operating hatching season that would closely approach the perfect reproductive performance commonly found in individual matings of the pedigree breeding flock.

Studies of both fertility and hatchability should be incorporated in the same research program whenever possible. Usually there appears to be no basic relationship between these two characters, but some significant positive correlations between them have occasionally been found (see chapters 3 and 7). Opposite trends in hatchability and fertility would not be expected to occur, such as an association of improved hatchability with lowered fertility. Yet it is conceivable that some practice that improved hatchability might have a deleterious effect on fertility. Obviously, both the poultry breeder and the hatcheryman are interested in obtaining simultaneous improvement in both characters.

ORGANIZATIONS FOR VARIOUS TYPES OF RESEARCH PROJECTS

An important responsibility of the individuals or agencies allotting funds for research in fertility and hatchability is the determination of the most favorable conditions for the solution of specific problems. Conversely, administrators of research laboratories have the responsibility of ascertaining that their organizations are devoted to the solution of the type of problem that can be handled best by the scientific experience and training of their staffs and by the equipment at hand. It is the writer's purpose to indicate some favorable conditions for various types of problems presented to the investigator by poultry breeders and hatcherymen.

Relatively Simple Problems

Not all problems of embryological development have the same characteristics. Some defective characters involve reactions that are alternatively expressed—either they appear or they do not—with no confusing intergradations. Such characters are readily produced in all embryos when the eggs have a certain constitution or are subjected to the required conditions. Some lethal genes, nutritional deficiencies, and reactions to faulty physical

conditions in incubation fall into this category. Any college or research laboratory staffed by a geneticist, nutritionist, or embryologist, respectively, should be prepared to work on problems of this kind. Many of the relatively simple problems of the hatcheryman could be solved in embryological laboratories provided that a reliable source of hatching eggs and dependable incubating equipment were available.

Complicated Problems

On the other hand, there are many defective characters or causes of poor hatchability that are not expressed with such exactitude as those discussed in the preceding section. The defects produced by more complicated determining factors are generally characterized by great variability in range of expression. The biochemical or biophysical conditions that favor the production of such defects are often complex; they frequently represent conditions existing in the breeding stock rather than in the hatchery.

This complicated type of problem demands the facilities of a well-equipped poultry experimental unit, including stock of known origin, trapnesting and pedigreeing of experimental eggs, controlled breeding diets, uniform management of the breeding flocks or incubators, and other controls. Such experimental work often deals with relatively slight differences in embryonic development produced by different treatments of the breeding stock or the hatching egg. Large numbers of eggs are frequently needed to establish the significance of the slight differences obtained in the hatching results. These differences, although small, may represent a considerable monetary loss for the poultry industry as a whole. This type of problem demands the facilities of an adequate plant and staff in an agricultural college, experiment station, or research department of an industrial firm. Research groups, composed of scientists trained in different fields of chemistry, physics, and biology, are better prepared to undertake this work than an individual investigator.

Under provisions of the Agricultural Research and Marketing Act of 1946, co-operation between the state experiment stations and agencies of the U. S. Department of Agriculture has been extended to the development of regional projects. The poultry-

breeding project of the North Central states and the turkey-breeding project of the Western states are examples of research work in which interstate groups of scientists deal with problems of such magnitude and complexity as could hardly be undertaken at a single college or experiment station. Both the breeding projects cited deal with reproductive characters, including fertility and hatchability. If effectively organized, the regional projects should be extended and serve to correlate the results obtained from work conducted at individual stations, and they may be expected at the same time to provide additional regional stations for testing and research work on a scale exceeding that of the larger state experiment stations.

Problems for Specialized Laboratories

Between the extremes of the simple and the complex, there are many variations in the nature of the fundamental problems involved. The existing facilities of governmental laboratories operating in public health work or of either public or private laboratories using the avian embryo in the production of vaccines or the testing of antibiotics may, as a by-product of their main research program, serve to increase our knowledge of the embryo. Information concerning the natural resistance of the embryo to different chemical or biological treatments may be derived from such laboratories. Other similarly specialized laboratories (see table 45) may also be considered potential sources of data on the embryology or physiology of the chick or poult.

Industry-Sponsored Research

Perhaps the most encouraging phase of nutritional, genetic, and disease-control research dealing with poultry is the financial support now being given by industrial organizations, poultry breeders, and hatcherymen to research projects. Programs of work on the factors influencing hatchability have received particular attention. Our knowledge of the nutritional requirements of breeding chickens and turkeys has been greatly advanced by results obtained in the laboratories of manufacturers of chemicals, pharmaceuticals, feedstuffs, and mixed feeds and by experiments conducted at colleges and experiment stations supported by grants from commercial firms. Similarly, but to a lesser degree, pro-

grams have been developed or sponsored in genetic studies of hatchability. Research in poultry pathology is now the subject of very active industrial support, particularly in the development of drugs and vaccines for the control of diseases under conditions existing on farms and in the hatchery.

The recognition that the economic losses sustained by the breeder and hatcheryman are of sufficient importance to warrant support by grants-in-aid from industry presents a hopeful outlook for future research in fertility and hatchability. Assistance from industrial associations, like the International Baby Chick Association which has sponsored the writing and publication of this review, can be expected to call forth an enthusiastic response from the administrators and scientists of research and educational institutions expressed in the training of men and women and in the formation of specific projects to solve the problems that are known today and those that will undoubtedly arise in the future.

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Supplementary References

Since the various chapters of this book were written, several publications have appeared that contain important new contributions to the subject of fertility and hatchability. Another book has become available in a new edition. The content of these reports and texts cannot be reviewed here, but the student of problems in this field should have reference to the following publications:

Biester, H. E., and L. H. Schwarte, 1948. *Diseases of Poultry*. 1154 pp.
Iowa State College Press, Ames, Iowa. (2d ed. of Biester and Devries,
1943.)

Eighth World's Poultry Cong. Official Rept., 1948, vol. I. 752 pp. Copen-
hagen.

Proc. 52nd Ann. Meet. U. S. Livestock Sanit. Assoc., Denver, Colorado,
October 13-15, 1948.

Romanoff, A. L., and A. J. Romanoff, 1949. *The Avian Egg*. 918 pp.
John Wiley & Sons, N. Y.

In addition to the above references, the journal *Poultry Science* has contained a number of papers on hatchability in volume 27 published during 1948. The student would be well advised to examine the current and future issues of this journal for further reports dealing with research in the general subjects of fertility, hatchability, and incubation.

Appendix

INCUBATION TROUBLE-SHOOTING CHART

(Prepared by W. M. Insko, Jr., J. E. Parker, E. M. Funk, and W. R. Hinshaw)

Symptoms	Probable Causes	Suggestions
Many clear eggs showing no development Infertiles	1. Too many or too few males 2. Seasonal decline in fertility in late summer and fall 3. Males undernourished as evidenced by poor fleshing and shrinking of comb and wattles 4. Interference of males during mating 5. Frozen comb and wattles during cold weather 6. Malea too old 7. Preferential mating—in pen matings 8. Sterility of males—usually in pen mating 9. Eggs held too long Eggs chilled by holding at too low a temperature	1. Use 1 male to 15 to 25 females with Leghorns and 1 male to 12 to 20 females with heavy breeds. Use 1 male to 10 to 12 females for turkeys. 2. Use early hatched cockerels 6 to 9 months of age depending on rate of sexual maturity. 3. Replace underweight males with vigorous males in good condition. Provide feeders on roosts. Dub Leghorn males. 4. Do not use too many males. Raise males together. Provide temporary partitions or blinds in large pens when breeders are confined. 5. Provide comfortable housing and use proper kind of drinking fountains. Dub males in cold climates. 6. Use cockerels instead of old males unless the latter are proved valuable breeders. 7. Artificially inseminate infertile hens or put with another male in different pen. 8. Replace with another male. 9. Set eggs within 7 to 10 days after laying. Hold eggs where the temperature is between 40° F. and 60° F.
Blood rings	10. Improper temperature 11. Improper fumigation 12. Holding eggs at temperatures above 80° F. before incubation Weakening embryos by chilling	10. Check accuracy of thermometer. Check thermostat, heating element, current supply; check operating temperature against manufacturer's instructions. 11. Do not fumigate at high concentrations during the first 5 days of incubation. 12. Hatching eggs should be held where the temperature is below 80° F., preferably below 60° F., and above 40° F.

Incubation Trouble-Shooting Chart

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INCUBATION TROUBLE-SHOOTING CHART (*Continued*)

(Prepared by W. M. Insko, Jr., J. E. Parker, E. M. Funk, and W. R. Hinshaw)

Symptoms	Probable Causes	Suggestions
Many dead germs	13. Temperature too high or low 14. Improper turning of eggs 15. Breeding (low hatchability inherited) 16. Improper ventilation—insufficient oxygen 17. Pullorum disease or other salmonelloses	13. See suggestions (10) above. 14. Turn at least 3 times, preferably 5 or more in 24 hours. 15. Avoid close inbreeding. 16. Increase ventilation of incubator and incubator rooms; avoid drafts. Add oxygen at high altitudes. 17. Use eggs from disease-free sources only.
Pipped eggs not hatching	18. Insufficient moisture	18. Increase evaporating surface for moisture or increase sprays. Chickens: first 18 days, wet-bulb: 85°-87° F.; 3-day hatching period, 90°-92° F. Turkeys: first 24 days wet-bulb: 87°-88° F.; 4-day hatching period, 90°-92° F.
Hatching too early Hatching too late Sticky hatch	19. Too high temperature 20. Too low temperature 21. Probably too high temperature	19-21. See (10) above. For all three—check temperature at maximum or when current actually goes off. During hatching period check temperature after current goes off to see if it increases further.
Malformed chicks Spraddlers	22. Temperature too high 23. Too low moisture 24. Improper turning or setting 25. Hatching trays too smooth	22. See (10) above. 23. See (18) above. 24. See (14) above. Set eggs large end up. 25. Use trays with wire or crinoline on bottom.
Abnormal chicks Weak chicks Small chicks	26. Overheating in hatching unit 27. Small eggs	26. See (10) above. 27. Set only standard or larger size eggs
Labored breathing	28. Insufficient moisture 29. Too much fumigant Respiratory disease (bronchitis or Newcastle)	28. See (18) above. 29. See under chapter 8 on Disease Control Measures. Check with nearest disease laboratory
Large, soft-bodied, mushy chicks, dead on trays, bad odor	30. Low average temperature 31. Poor ventilation 32. Navel infection (omphalitis) in incubator	30. See (10) above 31. See (16) above 32. Carefully clean and fumigate incubator between batches
Rough navels	33. High temperature or wide temperature variations	33. See (10) above.
Hatching too late or not uniformly.	34. Old eggs and eggs of different ages	34. Set eggs at least once each week

Glossary

(The following terms are defined in the restricted sense of use in this volume. Many of the words also have other meanings for use in other connections.)

ABERRATION. A deviation from the normal.

ACHONDROPLASIA. Chondrodystrophy.

ACROSOME. Sharp-pointed body on the front of the sperm which is believed to aid the sperm in penetrating the egg (ovum).

ADENYLPYROPHOSPHATASE. An energy-releasing enzyme which acts upon adenosine triphosphate (adenyl-pyrophosphate).

ADRENALIN. A hormone secreted in the medulla of the adrenal gland.

AGGLUTINATION TEST. A test based on the property of certain antibodies (agglutinins) to cause the clumping of bacteria when added to a suspension of them (antigen).

ALBUMEN. The white of the egg.

ALLANTOIC FLUID. The fluid contents of the allantois, composed in part of excretions from the embryonic kidneys.

ALLANTOIS. An outgrowth of the embryonic hindgut, which forms a highly vascularized membranous sheath in close proximity to the shell membranes of the egg.

ALLEL (ALLELOMORPH). One of two or more genes which are borne at the same locus of a chromosome.

AMINO ACID. The chief structural component of a protein, containing amino (NH_2) and carboxyl (COOH) groups.

AMINO NITROGEN. Nitrogen occurring as a constituent of the amino group (NH_2).

AMINOPEPTIDASE. An enzyme, or group of enzymes, in the small intestine, which acts on polypeptides containing a free amino group.

AMNION. A thin protective membrane forming a sac around the embryo.

AMNIOTIC FLUID. The watery fluid in which the developing embryo floats within the amnion.

AMYLASE. Any one of a series of enzymes which convert starch into sugar.

ANEMIA. A deficiency of red blood cells or of hemoglobin.

ANHYDRASE. An enzyme which catalyzes the removal of chemically combined oxygen and hydrogen as water.

ANTIBIOTICS. Substances derived from living organisms which destroy other organisms or inhibit their development.

ANTIBODY. A substance in tissue or blood that is specifically antagonistic to a toxin or poison.

ANTIGEN. A suspension of bacterial cells which is used in testing for the presence of antibodies in blood serum.

ANTISEPTIC. A substance that prevents or arrests the growth of micro-organisms.

ANTITRYPTIC. Counteracting the activity of the enzyme trypsin.

ANUS. The opening of the cloaca to the exterior of the body; the vent.

ARTIFACT. Any unnatural structure or change; used in histology and microscopy for a structure that has been mechanically altered from its natural state.

ASCITES. The collection of serous fluids in the cavity of the abdomen; dropsy.

ASPERGILLOSIS. Any disease caused by molds belonging to the genus *Aspergillus*.

ASYMMETRIC. Not symmetrical.

ATAXIA. Unco-ordinated muscular action.

ATYPICAL. Not typical; irregular.

AUTOSOMES. All chromosomes other than the sex chromosomes.

AUXIN. A hormone-like substance from sprouts of plants and from human urine which promotes growth in plant cells and tissues.

AVIAN. Of or pertaining to Aves or birds.

BACKCROSS. The crossing of a first generation hybrid with one of its parents or with an individual of similar genetic composition to a parent.

BACTERIA. A group of one-celled microorganisms to which belong a large number of the disease-producing germs.

BACTERINS. Suspensions of dead microorganisms (antigens) used to immunize animals against specific diseases; vaccines consisting of dead germs.

BIOCHEMISTRY. The chemistry of plant and animal life.

BLASTODERM. The circular mass of cells formed by early cell division in the germ spot or blastodisc.

BLASTODISC. The germinal spot or disc on the surface of the yolk.

BRONCHITIS. Any disease characterized by an inflammation of the bronchi (the two main branches of the windpipe).

CARBOHYDRATE. Any of a group of compounds composed of carbon, hydrogen, and oxygen in which the ratio of hydrogen to oxygen is 2 to 1; such as sugar, starch, cellulose, and dextrins.

CARBONIC ANHYDRASE. An enzyme which catalyzes the decomposition of carbonic acid into carbon dioxide and water.

CAROTENASE. An enzyme which converts carotene into vitamin A.

CATANOLISM. Destructive metabolism.

CATALYSIS. Acceleration of a reaction by a substance (called the catalyst) which may be recovered practically unchanged at the end of the reaction.

CATALYST. The agent in catalysis; called also catalytic agent or catalyst.

CATHEPSIN. A proteinase found in most cells, which takes part in cell autolysis (self-digestion of tissues).

CEPHALIC. Pertaining to or directed toward the head.

CHALAZA. A spiral band of thickened albuminous substances in the white of the hen's egg, extending from the chalaziferous layer toward each end of the egg.

CHALAZIFEROUS LAYER. A thickened albuminous layer of egg white enclosing and adhering closely to the yolk.

CHOLESTEROL. One of the most common sterols of animal origin.

CHONDRODYSTROPHY. A condition characterized by shortened extremities and crooked leg bones, resulting from abnormal ossification in cartilage.

CHROMOSOMES. Structures within the nucleus that bear the genes.

CILIA. Vibratile, microscopic, hair-like, protoplasmic projections of cells.

CILIARY BODIES. A ring of muscle tissue situated toward the front of the eyeball which controls the shape of the lens by exerting tension on the ligaments holding the lens in place.

CLOACA. The common cavity in birds into which the oviduct, the vasa deferentia, and the urinary and digestive tracts open.

CLUBBED DOWN. Imperfectly emerged down feathers.

CLUTCH. A series of eggs laid on successive days.

COLLAGEN. A glycoprotein from which gelatine is made.

CONCENTRIC. A series of circles of various sizes having a common center.

CONGENITAL. Existing at birth or hatching.

CONTROL. As applied to disease, the restriction of the spread of diseases by keeping the number of cases in an area reduced, but not completely eliminated; in experimentation, a normal standard of comparison to an experimental condition.

COPROPHAGY. The eating of fecal material.

COPROPORPHYRIN. A porphyrin formed in the intestines and found in normal feces; present in the urine in porphyrinuria.

CORYZA. An acute inflammation of the mucous membranes of the nasal passages; nasal catarrh; cold in the head.

CROSSBREEDING. Mating individuals belonging to different breeds or varieties; a form of outbreeding.

CRYPT. A glandular cavity.

CRYPTOXANTHIN. One of the carotenoid pigments that may be converted into vitamin A.

CUTICLE. A thin noncellular covering of the surface of the egg shell; also called the "bloom."

CYCLOPIA. An abnormal fetal condition in which a monster is formed with one median eye, or two eyes fused into one (cyclops).

CYTOCHROME OXIDASE (INDOPHENOL OXIDASE). An iron-containing oxidase present in various tissues and cells; a constituent of the respiratory enzyme system.

DDT (DICHLORO-DIPHENYL-TRICHLOROETHANE). An effective contact killer of many insects.

DEODORANT. Any substance which masks disagreeable odors or eliminates them entirely by removing their causes. A deodorant may or may not be a disinfectant or an antiseptic.

DERMATITIS. Inflammation of the derma or true skin.

DIALLEL CROSSES. A mating system in which two females are mated in turn to each of two males in order to evaluate the parental genotypes.

DIASTATIC. Converting starch into sugar by action of the enzyme diastase (amylase).

DILUENT. Any substance used to make another substance less concentrated.

DIPEPTIDASE. An enzyme that splits dipeptides into amino acids.

DISINFECTANT. A substance that destroys harmful microorganisms.

DISINFECTION. The act of disinfecting, or rendering harmful bacteria harmless.

DISTAL. Designating that end of a limb or other part that is farthest from the point of attachment.

DIVERTICULUM. A pouch or sac opening from any hollow organ or cavity.

DOMINANT. Designating in a heterozygous state the one of the two alleles that predominates in determining a particular characteristic.

DUBBING. Trimming of combs or wattles, or both.

ECTODERM. The outer of the three germ layers.

EDEMA. Abnormal accumulation of serous fluid in the tissues.

ELUTE. Material separated and removed by washing.

EMBRYO. An organism in the early stages of development; a bird prior to hatching.

ENCEPHALOMYELITIS. A disease of the central nervous system caused by a filterable virus; sleeping sickness.

ENTODERM (ENDODERM). The inner of the three germ layers.

ENZYME. A protein capable of producing by catalytic action the transformation of some other compound or compounds.

EPIDIDYMIS (pl. epididymides). An organ made up of many convoluted tubules located on the testicle and connecting the ducts of the testis with the vas deferens.

EPiphyseal Cartilage. The cartilage in the epiphysis, or head, of a long bone.

ERADICATION. As used in disease control, the complete elimination of a disease from a given area.

ERGOSTEROL. A plant sterol which is converted to vitamin D₂ by ultra-violet radiation.

ERYTHROCYTE. A red blood corpuscle.

ESTRIN. An estrogen produced by the ovary; follicular hormone.

ESTROGENS. A group of compounds, including the female sex hormone, which has a feminizing effect.

EXCURRENT DUCTS. Tubes leading out or away from an organ; efferent ducts.

FEMUR. The uppermost bone of the leg.

FERTILIZATION. Union of the sperm and the egg (ovum).

FETAL. Pertaining to the embryo.

FIBULA. The outer and smaller of the two bones of the leg which extend from the femur toward the tarsometatarsus; in birds this bone is often rudimentary.

FISTULA. An abnormal opening leading from an organ to the exterior.

FLAGELLUM. A long, slender, thread-like, undulating portion of a cell.

FOLLICLE. A membranous envelope enclosing the developing ovum or yolk.

FOLLICULAR HORMONE. A female sex hormone, estrin.

FORMALDEHYDE. A gaseous compound having the chemical formula HCHO ; it is a powerful disinfectant having a sharp, penetrating odor.

FORMALIN. A watery (aqueous) solution of formaldehyde. The commercial preparation has a strength of 40 per cent formaldehyde.

FUMIGATION. The act of applying a gas, vapor, or smoke as a means of disinfection.

GASTRULA. The stage of early embryonic development in which only the ectoderm and entoderm are present.

GENE. The basic unit of inheritance in the germ plasm; a hereditary factor.

GENOTYPE. The genetic composition of an individual.

GLYCOL (ETHYLENE GLYCOL). A thick colorless liquid with the chemical formula $\text{C}_2\text{H}_4(\text{OH})_2$. It is the basic part of numerous related alcohols in use as antiseptics and disinfectants.

GLYCOLYSIS. Hydrolytic decomposition of sugar.

GONAD. The primary reproductive organ of either sex; in the male, the testis or testicle and in the female, the ovary.

GONADOTROPIC. Stimulating the ovary or testis.

GONADOTROPIN. A hormone capable of stimulating the gonads.

HALLUX. The first or hind toe of chickens and turkeys.

HEMOGLOBIN. The red pigment of blood; the carrier of oxygen in the blood.

Hemophilus gallinarum. The bacterium that causes infectious coryza.

HETEROZYGOUS. Designating the presence of a pair of unlike alleles.

HISTOLOGY. The study of tissues of the body.

HISTOZYME (HIPPURICASE). An enzyme said to occur in the kidneys, capable of decomposing hippuric acid and its homologues.

Appendix

HOMOZYGOUS. Designating the presence of a pair of identical alleles.

HORMONE. A chemical secretion from a ductless or endocrine gland which stimulates or inhibits some organ of the body. Hormones are transported by the blood.

HUMERUS. The bone of the proximal part of the fore limb or wing.

HYBRID. The product of the mating of the male and female of two species or of individuals possessing dissimilar genotypes.

HYDROGEN-ION CONCENTRATION. A measure of the acidity or alkalinity of a solution, commonly referred to as pH. Solutions with pH 7 are neutral; with pH under 7, acid; and with pH over 7, alkaline.

HYDROLYSIS. A chemical process of decomposition involving addition of the elements of water.

HYPOPHYSECTOMY. A surgical operation for removal of the hypophysis (pituitary gland).

ICHTHYOLOGY. The science dealing with fishes.

IMMUNE. Completely resistant to a specific disease or diseases.

INANITION. Exhaustion from lack or nonassimilation of food, fasting.

INBREEDING. The mating of related individuals such as brother and sister, parent and offspring, and cousins.

INCINERATE. To burn to ashes.

INCROSSBREEDING. The crossing of inbred lines of different breeds.

INCROSSING. The crossing of different inbred lines within a breed.

INFUNDIBULUM. The most anterior section, the funnel, of the oviduct.

INSEMINATION. Introduction of semen into the female reproductive system.

INSULIN. The hormone of the pancreas, which promotes the utilization of sugar in the organism.

INTERSTITIAL CELLS. Cells located in the testis in spaces between seminiferous tubules.

INTERVAL. In egg production, the time intervening between the laying of successive eggs in a clutch.

INTRAPERITONEAL. Within the abdominal cavity.

INTRAVENOUS. Within a vein.

IODINE NUMBER. The number of grams of iodine taken up by 100 grams of fat; a measure of unsaturation.

ISTHMUS. The portion of the oviduct where the egg shell membranes are laid down.

KERATIN. A protein that forms the essential ingredient of horny tissue.

KINETIC. Pertaining to, or due to, motion.

LABORATORIAN. A person who operates a laboratory and is skilled in the techniques used therein.

Lactobacillus casei e. A bacterium used in the assay of certain vitamins.

LARYNGOTRACHEITIS. Any disease characterized by inflammation of the

larynx and trachea. Infectious laryngotracheitis is a specific disease of chickens, caused by a filterable virus.

LATEBRA. A flask-shaped mass of light-colored yolk leading from the center of the yolk to the germinal disc.

LECITHINASE. An enzyme that disintegrates lecithin.

LETHAL GENE. A gene that in the absence of a normal allele causes the death of an embryo or prevents it from hatching. Mutations with dominant lethal effects are self-eliminating.

LEUKOSIS. Any disease characterized by a proliferation of the precursors of leukocytes or "white" blood cells.

LIGAMENT. A band of tissue that connects or ties together related structures.

LINKED GENES. Genes which are borne on the same chromosome.

LIPASE. Any of a class of enzymes that accelerates the hydrolysis of fats to fatty acids and glycerol.

LIPOLYSIS (LIPOLYTIC). Decomposition of fat.

LUTEINIZING HORMONE. A hormone from the anterior pituitary which causes a corpus luteum to develop in the ruptured follicle of mammals after ovulation.

LYMPHOMA. A tumor made up of lymphoid tissue.

LYMPHOMATOSIS. A form of leukosis involving the development of multiple lymphomas in various parts of the body.

MACROSCOPIC. Visible to the unaided eye.

MAGNUM. The section of the oviduct that secretes the major portion of the mass of egg white; it extends from the infundibulum to the isthmus.

MAMMILLARY LAYER. The innermost layer of the egg shell.

MANDIBLE. The bone of the lower jaw or beak.

MAXILLA. A bone at the base of the upper beak.

MELANIN. Any of the various dark brown or black amorphous pigments of animal origin.

MESODERM. The middle germ layer of the embryo; the mesoblast.

METABOLISM. The chemical changes in living cells by which energy is provided for the vital processes and new material is assimilated.

METHYLATION. A process by which methyl groups (CH_3) are added to a compound.

METHYLENE BLUE. A dye that becomes blue on oxidation.

MICROMELIA. A general shortening of the wings and legs; dwarfism characterized by shortened extremities.

MITOSIS. Cell division involving equational division of chromosomes.

MONILIASIS. Any disease that is caused by the yeast-like microorganisms known as *Monilia*. In birds a disease of the crop mucous membrane, sometimes called "thrush."

MORPHOLOGY (MORPHOLOGICAL). The branch of biology dealing with form and structure.

MUCIN. A glycoprotein comprising the thickened portion of the egg white.

MUCOID. Any of a group of glycoproteins resembling true mucin, but differing in some reactions.

MUTANT. A sport or variation that breeds true.

MUTATION. A sudden variation in a gene or an inherited character.

MYELIN SHEATH. The sheath surrounding a nerve.

NEURAL TUBE. The epithelial tube developed from the neural plate and forming the central nervous system of the embryo.

NEWCASTLE DISEASE. An acute disease of fowls caused by a filterable virus and characterized by nervous and respiratory symptoms.

"NICKED." Where matings produce unanticipated, exceptionally superior offspring, the combination is said to have nicked.

NONVASCULAR. Lacking a supply of blood vessels.

NUCLEUS. A specialized structure in the cell that contains the chromosomes.

OFFAL. The waste parts of a butchered animal or bird, i.e., the entrails, feathers, head, feet, and shanks. Also called dressing loss.

OMPHALITIS. Inflammation of the umbilicus, or navel.

ONTOGENY. The life history or development of an organism.

OSSIFY (OSSIFICATION). To change into or form bone.

OTOCEPHALY. A condition in which the ears are approximated or fused below the face and the lower jaw may be missing.

OUTBREEDING. The mating of nonrelated individuals.

OUTCROSSING. Mating of unrelated individuals of the same breed or variety.

OVARY. The primary reproductive organ of the female; in domestic fowl normally only the left ovary develops.

oviduct. The tube through which the yolk passes to receive additions of egg white, shell membranes, and shell.

OVIPPOSITION. The act of laying an egg.

OVOFLAVIN. Rihoflavin.

OVOMUCOID. A mucoid present in egg white; a mucus-like substance derivable from egg white.

OVOMUCOIDASE. An enzyme which catalyzes the removal of glucose from ovomucoid.

OVOPORPHYRIN. The porphyrin of eggs.

OVULATION. The release of the ovum from the ovary.

OVUM. A nucleated cell formed in the ovary. In reference to the hen, the term usually applies to the cell product of the ovary along with a much larger mass of nutrients which comprise the yolk of the egg.

PALPATION. The process of exploring organs by touch; digital exploration.

PARTIAL PRESSURE. The pressure exerted by a constituent of air expressed in terms of millimeters of mercury.

PATHOLOGY. The science treating of diseases, their essential nature, causes, and development, and the structural and functional changes they produce.

PEPSIN. The proteolytic enzyme of the gastric juice, which changes the proteins of the food into proteoses and peptones.

PENISTALYSIS. A contractile, wave-like, explosive muscular movement of organs.

PENITONITIS. An acute inflammation of the membrane (peritoneum) that lines the cavity of the abdomen.

PEROXIOASE. An enzyme that promotes oxidation.

PHENOCOPIES. Environmentally produced imitations of hereditary characters.

PHENOL COEFFICIENT. A numerical factor designating the comparative efficiency of a disinfectant with phenol as a standard.

PHENOTYPE. The appearance of an individual with respect to a given character.

PHOSPHATASE. Any of a group of enzymes that accelerates the hydrolysis of organic phosphates.

PHOSPHOLIPID. Any of the complex lipids that contain phosphorus and nitrogen.

PHOTOPERIOOICITY. The response of an organism to the relative length of day.

PHYSIOLOGY. The study of functions of parts of the body.

PITUITARY. The endocrine or ductless gland located at the base of the brain; hypophysis.

PNEUMOENCEPHALITIS. A synonym for Newcastle disease of fowls.

POLYNEURITIS. A syndrome associated with the deficiency of thiamin.

PORPHYRIN. The iron-free nonprotein portion of respiratory pigments.

POTASSIUM PERMANGANATE. Dark purple crystals ($KMnO_4$) which are readily soluble in water and capable of liberating formaldehyde from formalin.

PRECURSOR. A substance that gives rise to another compound in the body.

PNEOVIPOSITAL. Before egg laying.

PROGYNON-B. A proprietary hormone preparation with feminizing properties.

PROTEASE. An enzyme that digests proteins.

PROTEOLYTIC. Effecting the digestion of proteins.

PROTHROMBIN (THROMBOGEN). The precursor of thrombin found in blood plasma.

PROTOZOA. The lowest division of the animal kingdom, including the unicellular organisms.

PROVITAMIN. A precursor of a vitamin.

PROXIMAL. Designating that end of a limb or other part which is nearest to the point of attachment.

QUICKLIME. Calcium oxide (burnt lime) which when exposed to air becomes "slaked" by absorbing carbon dioxide and water; used as a deodorant and a water absorbent.

R. (ROENTGEN). International unit of radiation.

RECESSIVE. Designating the allele that is not expressed in a heterozygous state; autosomal recessive genes must be in a homozygous state to produce their effect.

RESECTION. The surgical removal of a part of an organ.

SALICYLASE. An enzyme that oxidizes salicylaldehyde into salicylic acid. **Salmonella.** The generic name for the bacteria that cause paratyphoid infections (salmonellosis).

SAPONIFICATION. The chemical decomposition (hydrolysis) of a fat by alkali, forming a soap.

SAPONIFIED CRESOL SOLUTIONS. *Liquor cresolis saponatus*, or compound solutions of cresol. An official disinfectant containing 50 per cent (by volume) of cresol and soap prepared from 35 per cent linseed oil and alkali, which makes a brown liquid miscible with water.

SCIATIC NERVE. A nerve located in the region of the hip and extending to the thigh.

SEmen. Fluid produced in the male reproductive organs that contains the spermatozoa.

SEMINIFEROUS TUBULES. Small convoluted tubes that comprise much of the bulk of the testis and in which spermatozoa are formed.

SERTOLI CELLS. Cells located in the seminiferous tubules to which heads of maturing sperms are attached; often called nurse cells.

SEX CHROMOSOME. A chromosome determining sex.

SEX-LINKED. Genes located in the sex chromosome are referred to as being sex-linked, as are also characters determined by such genes.

SEXUAL MATURITY. The stage at which egg or sperm production commences.

SIB MATING. Mating of brother and sister.

SOMITES. Block-shaped masses of embryonic mesoderm that form a row on both sides of the neural tube, and later give rise to bone, muscle, and skin tissues.

SPERMATOGENESIS. The formation of male germ cells or sperms; the process by which primitive germ cells become spermatozoa.

SPERMATOXIN. A toxin destructive to sperm; antibodies acting against sperm.

SPERMATOZOA. Mature reproductive cells of the male; sperms.

STERILE. Infertile; incapable of reproducing.

STEROL. Any of a class of solid higher alcohols, widely distributed in plants and animals; cholesterol is the best-known member of the group.

STIGMA. A nonvascular band on the surface of the follicle of an ovum previous to ovulation.

SUCCINOXIDASE. An enzyme that catalyzes the oxidation of succinic acid to fumaric acid during the release of energy from carbohydrates.

SULFONAMINES. A term applied to the drug, sulfanilamide, and any of its derivatives.

SYNACTYLISM. Union of two or more digits.

SYNDROME. A group of signs and symptoms that occur together and characterize a morbid condition.

SYNERESIS. Separation of a liquid from a gel, caused by contraction.

SYNTHESIS. The art or process of making or "building up" a compound by the union of simpler compounds or elements.

TARSOMETATARSUS. The lowermost long bone of the leg; shank bone of chickens and turkeys.

TERATA. Monsters.

TESTIS (*pl. TESTES*). The primary reproductive organ of the male that produces the sperm.

THIOURACIL. A drug with thyroid-depressing properties, which slows down the rate of metabolism and induces fattening in poultry.

THYROID. A ductless, or endocrine, gland in the neck which secretes the hormone thyroxin.

TIBIA. The second leg bone from the hip, extending between the femur and the tarsometatarsus.

TITER. In agglutination tests, the highest dilution of a serum at which clumping of the antigen occurs.

TOM. A male turkey.

TOINCROSSING. The crossing of inbred males with unrelated noninbred females.

TYROSINASE. An oxidizing ferment in animal tissues that converts tyrosine into pigments similar to melanin.

ULNA. The post axial or inner one of the two bones of the fore limb extending between the humerus and the bones of the wrist.

ULTRAVIOLET LIGHT. Light rays with wavelengths shorter than those of visible light. These rays are a substitute for vitamin D and have disinfecting properties.

UREASE. An enzyme that accelerates the hydrolysis of urea into ammonium carbonate.

UTERUS. The section of the oviduct where the egg shell is formed.

VACCINATION. The protective inoculation with a vaccine for the purpose of increasing the resistance against a specific disease.

VAGINA. The section of the oviduct connecting the uterus and the cloaca.

VASCULAR. Supplied with or containing blood vessels.

VAS DEFERENS (*pl. VASA DEFERENTIA*). The excretory duct of a testicle; spermatic duct.

VIRUS. A microorganism that is too small to be visible by aid of the compound microscope.

VITELLINE MEMBRANE. A very thin membrane enclosing the yolk.

WOLFFIAN BODY. The second embryonic kidney of the avian embryo; mesonephros.

XANTHOPHYLL. A yellow pigment usually associated with chlorophyll and carotene in plants; it colors such animal fats as egg yolks.

YOLK SAC. A more or less spherical sac attached to an embryo and enclosing the food yolk.

ZYGOTE. A fertilized egg; the individual developing from such a cell.

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twice as great; hence, the total number of sperms produced is comparable in the two species.

Results of experiments on the relation of the body conformation of male turkeys to semen production do not appear in the literature. In view of the popularity of Broad Breasted Bronze turkeys and the difficulty often encountered with fertility in this variety, it would seem that such research might yield results highly beneficial to turkey breeders.

Fertility in the Female Turkey

Few experiments have been conducted on factors affecting fertility in the female turkey and some of those that have been reported were not comprehensive. The number of sperms required to produce optimum fertility in the turkey hen is not definitely known. Parker (1946b) presented evidence that the number of sperms required for optimum fertility in turkeys may be less than the 100 million required for chickens as reported by Munro (1938d), since subsequent fertility of eggs was not noticeably affected when as few as 37 to 48 million sperms were inseminated.

Apparently turkey hens remain fertile longer after mating than do chicken hens. Burrows and Marsden (1938) observed that there was not much difference in the percentage of fertility when hens were inseminated at intervals of 1, 2, 3, and 4 weeks. Turkey hens inseminated at 30-day intervals laid eggs which were 83 per cent fertile. Under natural mating conditions, Scott (1937) found no appreciable reduction in the fertility of eggs 13 days after the removal of the males, and 40 per cent of the eggs laid on the thirty-fourth day after removal were fertile. Marsden and Martin (1946) observed fertile eggs for as long as 59 days after mating. After artificial insemination, Lorenz (1947) found that the percentage of fertile eggs laid declined from a peak of 96 per cent on the fourth day to 69 per cent on the thirty-fourth day and then dropped sharply (fig. 33). The last fertile eggs were laid 50 days after insemination.

That broody hens may lay a higher percentage of fertile eggs than nonbroodies was indicated by studies reported by Parker and Barton (1945a). They observed that hens in natural matings that were broody 1 to 4 times in the season laid eggs aver-

aging 92 per cent fertile, whereas eggs from nonbroody hens were 82 per cent fertile. It should be pointed out, however, that in spite of their lower fertility the nonbroodies produced more poulets per hen because of higher egg production. Margolf, Harper, and Callenbach (1947) noticed increased mating activity during

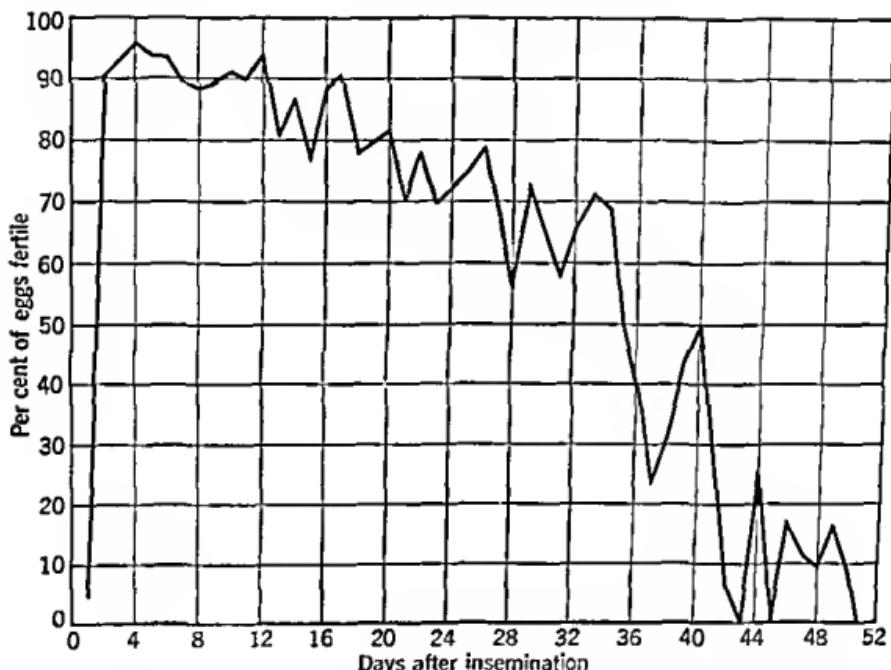


FIG. 33. Duration of fertility in turkey hens after one insemination with 0.05 cubic centimeter of semen. A total of 2103 eggs from 61 hens was involved. Courtesy F. W. Lorenz, Univ. California.

periods of increased broodiness which was due in large measure to resumed mating by broken-up broody hens. More recently Jones and Kohlmeyer (1947) reported that turkey hens with either pause or broody periods were no more fertile than continuous layers.

Although the afternoon insemination of chicken hens is conducive to highest subsequent fertility, it has not been shown that the time of mating affects fertility in turkeys. Limited data on the subject by Parker and Barton (1946) showed that the difference in fertility of eggs from hens artificially inseminated in the morning and in the afternoon was too small to be significant.

Fertility in Matings of Turkeys

Some interesting studies on the sexual behavior of White Holland turkeys were reported by Margolf, Harper, and Callenbach (1947). They observed that the number of times each hen mated ranged from 1 to 16 before egg production and from 4 to 40 for the entire breeding season. Frequency of mating, either before onset of production or during the breeding season, had no con-

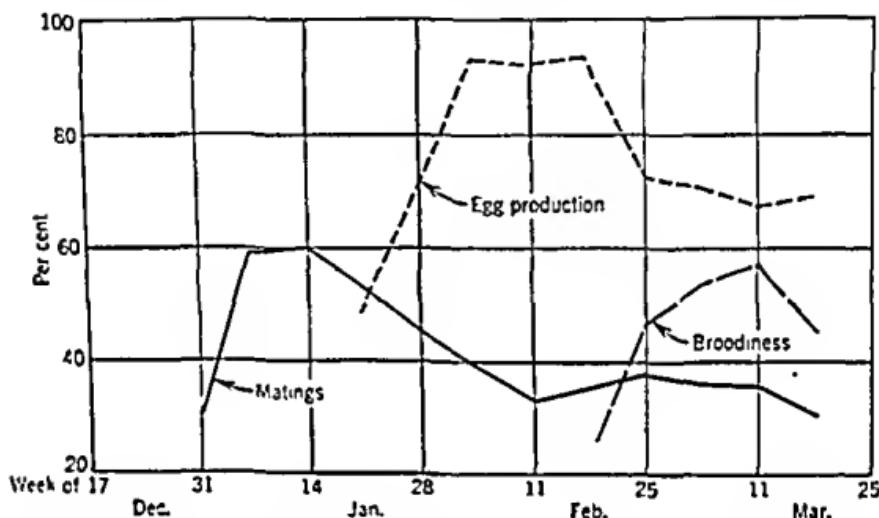


FIG. 34. Relation of mating frequency of White Holland turkeys to egg production and broodiness. Lighting commenced on toms December 10 and on hens December 21. Courtesy Pennsylvania Agr. Expt. Sta.

Breasted Bronze turkeys have been observed by Parker (1947) in North Dakota and by Harper (1947) in Oregon. Both found that fertility was high during February and March after which Harper observed a consistent decline during April and May (table 6). Parker did not notice this seasonal decline in fertility

TABLE 6

SEASONAL CHANGES IN FERTILITY OF TURKEYS

(Data on 159 Broad Breasted Bronze turkey hens in flock matings, Harper, 1947)

Date	Eggs set	Per cent fertile
Feb. 16	941	80.2
Feb. 26	1062	85.3
Mar. 8	900	85.6
Mar. 18	753	87.3
Mar. 28	537	85.1
Apr. 7	477	82.4
Apr. 17	499	81.2
Apr. 27	568	80.6
May 7	551	77.1
May 17	535	74.0
Season	6823	82.4

until May and June. Marsden and Martin (1946), reporting on a commercial flock for which reproduction data were kept on a year-round basis, showed that fertility tended to increase from August to a peak in March, after which there was a decline through July.

Although in Oklahoma Milby and Thompson (1945) reported that a cold wave of 10 days' duration caused a marked decline in fertility in all varieties, Parker and Barton (1945b) and Parker (1947) in North Dakota observed the highest fertility of the season in February when temperatures were as low as -18° F. It is conceivable that sudden drops in temperature affect fertility more than does actual coldness.

Preferential mating has been reported by Wileke (1939) and by Milby and Thompson (1945) as responsible for a great deal of low fertility in some matings, particularly in single-male pens. Neither Wileke nor Milby and Thompson (1942) found that arti-

ficial lighting had any consistent effect upon the fertility of the eggs laid.

Relatively little information is available on the inheritance of fertility in turkeys. Asmundson (1941), using females, and Lorenz and Lerner (1946), using toms, presented evidence that the age at which sexual maturity is attained is influenced by heredity. Comprehensive studies on the effect on fertility of crossbreeding turkeys were reported by Clark, Runnels, and Livesay (1944). Reciprocal crosses were made using Bronze, Bourbon Reds, and Broad Breasted Bronze as well as three-way crosses and backcrosses. The results revealed that crossbreeding did not affect fertility significantly, although there was an average difference in favor of the crossbreds of 5.3 per cent. By means of a system of outbreeding, Marsden and Knox (1937) were able to maintain a high level of fertility for 4 years with Bronze turkeys. It was also observed that intensive inbreeding adversely affected fertility, whereas less intense forms of inbreeding had less effect on fertility (table 7).

TABLE 7

INFLUENCE OF INBREEDING ON FERTILITY OF BRONZE TURKEYS
 (Data from Marsden and Knox, 1937)

Degree of inbreeding	Coefficient of inbreeding	Percentage of fertility
Outbred	0.000-0.063	87.8
Mild	0.125-0.218	75.4
Close	0.250-0.411	82.8
Intensive	0.500-0.672	69.3

Breeders of Broad Breasted Bronze turkeys put considerable emphasis on balance or carriage in the selection of their breeding males. Thompson (1943) included in his program the selection of breeding males with good conformation and carriage and obtained high fertility from Broad Breasted Bronze turkeys.

Confinement of breeding turkeys and the rotation of toms at weekly intervals in single-tom matings had little or no influence on the fertility of eggs, according to Parker and Barton (1946). Additional experimental data on the many existing methods of managing turkey breeding stock will be required before sound recommendations relative to mating turkeys can be made.

ARTIFICIAL INSEMINATION OF CHICKENS AND TURKEYS

The subject of artificial insemination may include factors influencing semen production in the male and fertility in the female as well as the technique of artificial insemination. Since the two former phases have already been discussed, the following paragraphs will for the most part review literature closely related to the technique of artificial insemination.

Collection of Semen

Investigators studying avian semen have employed various means of collecting it. Payne (1914) and Craft, McElroy, and Penquite (1926) collected semen from the cloaca of the hen after mating, and Hutt (1929) intercepted ejaculates with a watch glass during mating. An apparatus made of wire and animal membrane was fitted over the cloaca of the female by Ishikawa (1930) as a means of collecting semen. Another somewhat similar device which fitted around the vent of the male was described by Parker (1939).

An electrical method of stimulating ejaculation of birds was reported by Sercbrovsky and Sokolovskaya (1934). Electric shocks were induced by placing the positive pole in the skin in the sacral region and the negative pole in a basin of water into which the beak was immersed. Usually about four 80-volt shocks, of 3- to 4-seconds duration at 1- to 2-second intervals, were sufficient to induce ejaculations.

The methods of collecting semen described above are of interest historically but, since a more expedient and positive method of collecting semen was introduced by Burrows and Quinn (1937, 1939a), they have little place in routine artificial insemination. When semen is collected by the Burrows and Quinn technique, the male is held with a thigh in each hand by one of the two operators (fig. 35). The thighs should not be held too tightly. The second operator pushes the tail of the male forward with the palm of the left hand and then massages the abdominal region below the pubic bones with light but rapid strokes with the right hand.

When the bird has been stimulated to protrude the copulatory apparatus the semen is squeezed or milked from the bulbous ducts (fig. 36) by pressing the thumb and forefinger of the left hand together around the vent. The semen may be collected in various receptacles. Burrows and Quinn used a small glass funnel the



FIG. 35. Stimulating the male to protrude the copulatory organ by Burrows and Quinn technique. *Courtesy U. S. Dept. Agr. and Poultry Science.*

stem of which was plugged with paraffin and stuck in a rubber stopper. A tablespoon or whiskey jigger is also satisfactory.

Semen may be collected from turkey toms by the technique described above (fig. 37). Heavy toms can be held better when the person holding the bird assumes a stooped position; in such a position much of the weight rests upon the knees and the thighs of the holder. It is considerably more difficult to collect semen from turkey toms than from male chickens. A third person to collect the semen from turkeys is desirable, although it is possible for two men to do the job.

Male chickens may also be stimulated by placing the left hand on the bird's back and sliding the thumb and fingers backward toward the base of the tail. Stroking the males in this manner often causes protrusion of the copulatory apparatus, and it is but a step further to extend the thumb and forefinger around the base of the tail and milk the semen.

Since this manipulation is accomplished with the left hand the right hand is used to hold the male. By placing the collecting receptacle at a convenient height the entire operation may be performed by one person. This technique was also described by Burrows and Quinn (1937).

In the collection of semen from chicken males it is necessary to segregate the males from the females, and it is also desirable to separate the males from each other. It is difficult to obtain semen from males running with females. Although semen may often be collected from turkey toms in the breeding pen, greater volumes are obtained if toms are away from the hens. It is not necessary to keep toms in individual cages.

The volumes of semen collected from male chickens average between 0.5 cubic centimeter and 1.0 cubic centimeter, and those from turkey toms about half these amounts (table 3, page 108), depending on the age or breed of males, season of year, and other factors that influence semen production. In the collection of chicken semen for the purpose of artificial insemination, it is probable that daily collections yield the greatest numbers of sperms (Burrows and Quinn, 1939a; Parker, McKenzie, and Kempster, 1942a). When the interval between two successive collections varied from 30 minutes to 8 hours, Warren and Gish



FIG. 36. Collecting semen from male domestic fowl. Courtesy Oregon State College.

(1943) observed little variation in semen volumes. To obtain maximum volume of semen from a male at a particular time, two collections 15 to 30 minutes apart may be made.

Unlike semen from most farm animals, avian semen has not been stored satisfactorily for any length of time. Although mo-



FIG. 37. Collecting semen from the turkey tom. *Courtesy Oregon State College.*

tility of the sperms persists longer when sperms are stored at lower temperatures (1° to 10° C.), fertilizing capacity is lost sooner than at higher temperatures. Burrows and Quinn (1939b) reported that when semen was stored at 20° C. (68° F.) it retained its fertilizing capacity for as long as 8 hours, and Warren and Gish reported good results with semen stored for 7 hours at 50° F.

Although a large number of diluting fluids have been studied in their relation to duration of fertility, only a very few have been

investigated in relation to their influence on the sperms' capacity to fertilize eggs. Munro (1938d) observed that a synthetic diluent (Milovanov's solution) supported motility under storage conditions but adversely affected the fertilizing capacity of the sperm in proportion to the rate of dilution. Sperm serum, on the other hand, produced little harmful effect when used as a diluent. Using Ringer's solution as a diluent, Bonnier and Trulsson (1939b) observed that suspensions of 10 and 30 per cent of semen gave as good results as undiluted semen, whereas 2 per cent of semen gave poor results.

Additional research directed to finding means to increase the time during which avian semen can be effectively stored would extend the usefulness of artificial insemination.

Insemination of Females

Most of the work with artificial insemination of poultry before 1936 was unsuccessful because the semen was inseminated into the cloaca instead of directly into the oviduct. Quinn and Burrows (1936) described a technique of artificially inseminating hens in which the oviduct was extruded through the vent and semen was injected directly into the vagina or uterus. In order to extrude the oviduct, the hen is held with the left hand around the keel or breast. She is then placed in a head-downward position with her back against the operator's abdomen. While the hen is held firmly against the operator's body, the left thumb and forefinger are used to exert pressure around the vent (fig. 38). It is the sudden and simultaneous pressure exerted by both hands that causes the oviduct to protrude.

The method for extruding the oviduct of the turkey hen is similar to that for chickens described above, except that, on account of the size of the turkey, the bird's head is placed between the operator's legs and its breast is supported on the sloping lap. Both the right and the left hands are placed around the vent and pressed downward simultaneously to extrude the oviduct (Burrows and Quinn, 1939a).

After the oviduct is extruded the semen is injected directly into it (figs. 38 and 39) by means of a syringe. All pressure should be released from the hen's body before the plunger of the syringe is forced. As the oviduct returns to normal position a constant

light pressure on the syringe is necessary in order to prevent the oviduct from retracting from it.

In some turkey hens the entrance into the oviduct is closed by an occluding plate or hymen (Asmundson, Lorenz, and Moses, 1946). This is especially true during the early part of the breeding season before the onset of egg laying. The occluding plate



FIG. 38. Artificially inseminating a female chicken. Note the extension tube fitted to the syringe with piece of rubber tubing. *Courtesy Tennessee Agr. Expt. Sta.*

in the chicken has been described by Kar (1947). According to Greenwood (1935) the organ is usually destroyed before pullets lay their first egg.

An intraperitoneal method of inseminating hens by which semen is injected into the body cavity of hens in the vicinity of the ovary has been described by Van Drimmelen (1945a). A certain degree of skill is required in this method to prevent rupture of yolks or injury to large blood vessels.

As has been previously mentioned, the presence of a hard-shelled egg in the oviduct interferes with fertility and for that reason chicken hens should be inseminated in the afternoon after

most of the hens have laid (Moore and Byerly, 1942; Warren and Gish, 1943).

In experiments with chickens in which the dosages of undiluted semen varied from 0.2 to 0.02 cubic centimeter, Burrows and Quinn (1938) found that the percentages of fertile eggs subsequently laid decreased when less than 0.05 cubic centimeter of



FIG. 39. Artificially inseminating a turkey hen. Courtesy U. S. Dept. Agr.

semen was inseminated. Larger dosages resulted in very little, if any, increase in fertility. The relation of the amount of semen artificially inseminated to fertility in turkey hens was reported by Parker (1946b). Fertility of eggs was not affected a great deal when the amount of the dose of semen varied from 0.005 to 0.1 cubic centimeter of undiluted mixed semen and the inseminations were made at 3-week intervals.

When chicken hens were inseminated twice and three times weekly, Burrows and Quinn (1938) and Black and Scorgie (1942) observed that fertility was higher than when inseminations were made at weekly intervals. Weekly inseminations, however, gave

better results than inseminations made at 2-week intervals. On the other hand, when turkey hens were inseminated at intervals of 1, 2, 3, and 4 weeks little difference in the fertility of the several groups was observed by Burrows and Marsden (1938).

It has been shown by Burrows and Quinn (1938) that good technique and some experience on the part of the personnel are required to obtain highest fertility from artificial insemination.

Uses of Artificial Insemination

It has been pointed out by Burrows and Quinn (1939b) and Bonadonna (1939) that artificial insemination offers poultrymen a practical means of a more economical use of valuable or proved sires, a solution to the fertilization of hens in batteries, a useful tool in certain types of experimentation, and a way to make crosses between certain breeds and species in which natural matings are difficult or impossible. Warren and Gish (1943) pointed out that artificial insemination can be of definite value where a breeding male in a single-male pen must be replaced because of mortality or incapacity. It reduces the overlapping of the chicks sired by the two males. Warren and Gish further indicated that artificial insemination probably offers the greatest promise when used in connection with the progeny test, because it permits changing the mates of males during the breeding season with little or no loss of time and without substantially affecting the accuracy of the pedigree records. Such a procedure would allow each male to be mated with a greater number of females and would also permit each female to be mated with two or more males during the breeding season. The advantages of such a program are obvious to the specialized poultry breeder.

Jeffrey (1945) cited a commercial breeder who had used artificial insemination successfully in making progeny tests on cockerels by mating them to hens confined in laying batteries. The writer has used artificial insemination for several years in a pedigree breeding program with chickens kept in laying houses. Such a system of mating obviates the numerous small pens required for single-male matings, because all the females mated to a number of males may be maintained together in one large unit. All females that are mated to one male artificially are marked with leg bands of one color or of a common number. Prior to

insemination the hens are caught and each male's "mates" are placed in a separate coop or battery compartment.

The possibility of using artificial insemination on some of the low-fertility strains of turkeys should not be overlooked. There is little doubt but that artificial insemination would increase fertility in many flocks of breeding turkeys—especially if the artificial insemination was used to supplement natural mating.

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CHAPTER 4

The Care of Hatching Eggs before Incubation

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INTRODUCTION

Hatcherymen know that the hatchability of eggs may be reduced or completely destroyed by improper care of the eggs before they are incubated. The improper care of hatching eggs causes an estimated loss of several million dollars annually. The exact cause of this loss is sometimes difficult to determine. It is the purpose of this chapter to review the work that has been done to prevent such losses and to suggest a program for the proper care of hatching eggs.

TEMPERATURE

That the temperature at which hatching eggs are held before incubation is important has long been recognized by investigators and by those who incubate eggs. Exposure to either high or low temperatures will soon destroy the hatchability of an egg.

Minimum Temperature for Embryonic Development in the Domestic Fowl

The temperature at which development of the chick embryo is initiated is of practical interest to poultrymen and hatcherymen because it determines the temperature below which eggs must be held before they are incubated. The physiological zero point of the chicken egg (that is, the temperature at which the chick begins to develop) was reported by Prevost and Dumas (1825) as 29° C. (84.2° F.), by Dreste (1891) as 28° C. (82.4° F.), by Kacstner (1895) as 28° C. (82.4° F.), and by Edwards (1902), as from 20.0° C. to 21.0° C. (68.0° F. to 69.8° F.). A statistical analysis of Edwards' work, however, does not substantiate his conclusion that embryonic development is initiated at from 68.0° F. to 69.8° F. His data show that the blastoderm was not enlarged at 77° F. Edwards did not know the exact history of the eggs he used, and some preincubation may have occurred before the eggs were received at his laboratory.

Funk and Biellier (1944), working on the problem of egg quality, found that enlargement of the blastoderm did not occur at 70° F. or at 76° F. In a series of tests to determine the temperature at which the blastoderm of hens' eggs was enlarged they found, as shown in figure 40, that there was no appreciable development until the temperature was raised above 80° F., but that growth was very rapid at 85° F.

From the work done to date it is evident that embryonic development sufficient to be measured macroscopically does not occur until the eggs are held at temperatures above 80° F. Very rapid growth is initiated between 80° F. and 85° F.

Effect of Low Temperatures on the Hatchability of Eggs

In 1923 Mauro, an Italian investigator, reported that the hatchability of eggs held in a refrigerator at 32.9° F. for 24 hours was